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The Influence of Zostera marina and Ruppia maritima on Habitat Structure and Function in a Changing Environment in the Chesapeake Bay

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The Influence of *Zostera marina* and *Ruppia maritima* on Habitat Structure and Function in a Changing Environment in the Chesapeake Bay

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A Thesis

Presented to

The Faculty of the School of Marine Science

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In Partial Fulfillment

of the Requirements for the Degree of

Master of Science

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by

Emily D. French

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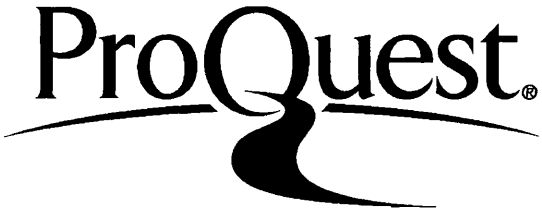

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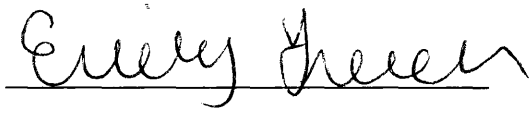

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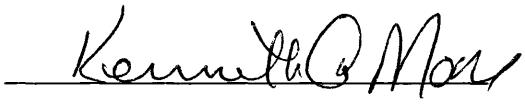

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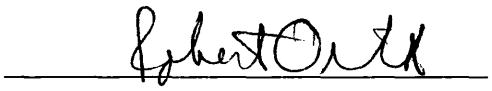

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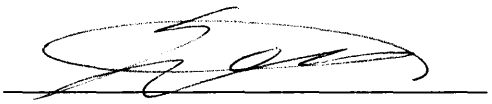

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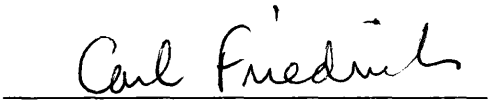

Carl Friedrichs, Ph.D.

TABLE OF CONTENTS

Page

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LIST OF TABLES

Chapter 1

Chapter 2

LIST OF FIGURES

Chapter 1

Figure **Page**

Chapter 2

Figure **Page**

THESIS ABSTRACT

Seagrasses modify the coastal areas they occupy by trapping sediments and improving water clarity, providing habitat for marine animals, and cycling nutrients. Populations are in decline worldwide, and in the lower Chesapeake Bay, U.S., *Zostera marina* populations are decreasing due to poor water quality and high summertime temperatures. *Ruppia maritima*, a seagrass that is smaller, but has a greater tolerance of high temperatures than *Z. marina*, is replacing *Z. marina* in some areas. This study examined bed characteristics and microbial community structures of each seagrass species, as well as mixed assemblages, at three sites in the lower Chesapeake Bay where *R. maritima* has been replacing *Z. marina* over recent years. The objective of this study was to determine the potential of *R. maritima* to ameliorate detrimental effects of *Z. marina* loss. Samples were taken in June and August of 2013. In contrast to expectations that *R. maritima* would increase in abundance by August, *R. maritima* biomass and density decreased. Sediment grain size showed interactions between site and habitat type; two sites of the three showed greater mean fine sediments in *Z. marina* than *R. maritima* stands. Where sediment erodibility was measured, eroded mass was greater in the *Z. marina* sediment compared to the *R. maritima* sediment in June, while eroded mass was greater in *R. maritima* sediment in August. This suggests that sediment trapping capabilities may differ seasonally between the two species, with *Z. marina* generally capable of trapping more fine sediments than *R. maritima*; however this capability may be affected by location and season. *Z. marina* provided better quality habitat for epifauna in the early summer, but results from late summer were inconsistent as both species died back. Microbial communities, which affect sediment nutrient cycling, were found to be similar among sediments occupied by both species of seagrass, although the effects of site and month were strong. There was also a greater relative abundance of sulfate reducers in the August samples than the June samples. Overall, the results demonstrate that although the quality of some ecosystem services were greater in *Z. marina* compared to *R. maritima*, *R. maritima* still possessed the ability to provide valuable ecosystem services, and could be considered as a restoration option in the Chesapeake Bay, especially in areas where the potential for *Z. marina* regrowth is low.

CHAPTER 1:

Sediment Trapping and Habitat Characteristics of *Zostera marina* and *Ruppia maritima*

ABSTRACT

In shallow coastal habitats, seagrasses modify the environment by baffling currents and anchoring sediments, and their canopies provide habitat for marine animals. In the lower Chesapeake Bay, the historically dominant *Zostera marina* is dying back due to poor water quality and high summertime temperatures, and is being replaced in some areas by native *Ruppia maritima*, which has a greater tolerance for high temperatures. *Z. marina* is a more robust species and has greater biomass per unit area than *R. maritima*. Because a continued decline of *Z. marina* with replacement by *R. maritima* could result in changes in ecosystem services, plant biomass, epifaunal assemblages and sediment dynamics were studied at two intervals during the summer of 2013, at three sites where both species occur and *R. maritima* abundance has been expanding. The objective of this study was to analyze the ability of *R. maritima* to ameliorate the detrimental effects of *Z. marina* loss. At two of the three sites evaluated, there were more fine sediments inside *Z. marina* dominated areas than *R. maritima* dominated areas, suggesting *Z. marina* is more effective at trapping sediment than *R. maritima.* In June at two of the three sites, *Z. marina* contained more epifauna than *R. maritima*, suggesting that *Z. marina* provides a better habitat than *R. maritima* in the early summer. In August however, when *Z. marina* typically dies back and *R. maritima* increases, epifaunal abundances become inconsistent. Although the quality of ecosystem services of *Z. marina* may be higher than that of *R. maritima*, *R. maritima* still possesses the ability to trap sediment and provide habitat, and should be considered as a partial replacement for *Z. marina* and a potential Chesapeake Bay seagrass restoration option.

INTRODUCTION

Seagrasses are marine flowering plants, and are unique features of coastal habitats that serve many essential roles in these ecosystems. Their canopies provide habitat, protection and sustenance for a wide variety of marine life, including small crustaceans that are prey of higher trophic levels (Douglass et al. 2010). Seagrass blades attenuate wave energy (Fonseca and Calahan 1992) and reduce current velocity (Fonseca et al. 1982), which leads to more fine particles being deposited and retained in seagrass beds (Ward et al. 1984). This process improves water quality by removing particles from the water column, creating a positive feedback loop for seagrass growth (van der Heide et al. 2007).

Seagrasses have been referred to as 'coastal canaries' (Orth et al. 2006) because they are valuable indicators of ecosystem health and are very susceptible to environmental change. Seagrasses are different from terrestrial plants in that light required for their growth and survival is attenuated not only by the water overlying it, but also by particles that reflect, absorb, and scatter light within the water (Dennison et al. 1993). Therefore, alterations in water quality are one of the biggest threats to healthy seagrass. Photosynthesis decreases when seagrasses are exposed to less light then they are acclimated to, which keeps the plant from manufacturing the resources needed for survival, and also slows the flow of oxygen from the roots, which can lead to sediment toxicity and die back of the plants (Holmer and Bondgaard 2000). Detrimental effects of light reduction can be further exacerbated when seagrasses are under temperature stresses, for example, during a particularly hot summer (Moore and Jarvis 2008).

Due to their nature of establishment in coastal areas, which have the highest densities of human population and subsequent sediment and nutrient loading, seagrasses worldwide are declining (Orth et al. 2006). The major causes for decline are the direct impacts from coastal development and dredging, and the indirect impacts of declining water quality, all of which block light to seagrasses (Waycott et al. 2009). Anthropogenic nutrient loading enhances growth of phytoplankton, macroalgae, and epiphytic algae that grows on the surface of blades (Cardoso et al. 2004). Turbidity can become elevated as a result of dredging, clearing land, and storms (Longstaff and Dennison 1999), and also increased nutrient supply and consequent algal growth (Cardoso et al. 2004).

With environmental changes, species that are either tolerant of degraded environments, or have a wide range of environmental tolerances, can increase in abundance. In many regions of the world, seagrass ecosystems are vulnerable to regime shifts from seagrass to macroalgal dominance (van der Heide et al. 2007, Thomsen et al. 2012) due to worsening water quality and temperature stress associated with climate change. Because macroalgae is responsive to nutrient inputs, it may bloom and compete with seagrass for resources such as light. When ecosystems experience a regime shift from seagrass to macroalga, the potential for sediment trapping and functionality as habitat for epifauna is dampened (Tuya et al. 2014, Cardoso et al. 2004).

In many seagrass ecosystems, species fall into the categories of dominant or opportunistic. Opportunistic seagrasses can grow quickly and have ephemeral populations, with less robust above and below-ground material than their dominant counterparts, which can occur in beds that persist for many years (Lopez-Calderon et al. 2010, Cho et al. 2009, Johnson

et al. 2003, Fourqurean et al. 1995). Environmental changes such as increased water temperatures, light reductions, or a combination have the potential to create conditions whereby dominant seagrass species decline. Opportunistic seagrass species may be more tolerant of these degraded conditions, and may even expand into areas formerly occupied by the dominant seagrass (Bologna et al. 2007, Johnson et al. 2003, Micheli et al. 2008).

Although there have been several instances of opportunistic seagrass species replacing dominant seagrass species (Fourqurean et al. 1995, Micheli et al. 2008, Lopez-Calderon et al 2010, Johnson et al. 2003, Moore et al. 2014, Bologna et al. 2007), some of these events have only lasted a season or several seasons before recovery of the dominant species, coincident with water quality improvements (Bologna et al. 2007, Johnson et al. 2003). Opportunistic and dominant species may also recover at the same time from environmental changes that had negative effects on all seagrasses in the area (Vaudrey et al. 2010), and additionally, it has been proposed that opportunistic seagrass species may modify the environment favorably, facilitating conditions for the dominant species to return (Cho et al. 2009). Whether the replacement of dominant seagrass species with opportunistic species is permanent or seasonal, it is important to study functionality of seagrasses in order to determine what ecosystem services changes to expect during and after these periods of replacement.

Seagrasses in the Chesapeake Bay

In the Chesapeake Bay, the largest estuary in the U.S., *Zostera marina* and *Ruppia maritima* are the two seagrasses that occupy the polyhaline region (Orth and Moore 1984). Typically in this region, *R. maritima* grows in shallower depths than *Z. marina*, while *Z. marina* is

more abundant at greater depths, although both can co-occur at intermediate depths (Orth and Moore 1988). Additionally, *Z. marina* reaches its greatest biomass in early summer, while *R. maritima* reaches its greatest biomass in late summer (Moore et al. 2000). These spatial and temporal differences are related to the higher light requirements for *R. maritima* (Wetzel and Penhale 1983) as well as its greater photosynthetic capacities at high water temperatures (Evans et al. 1986); so the periodicities of the two species make them well adapted to co-exist in this region (Orth and Moore 1988). *Z. marina* is typically the dominant species; it has a taller canopy and greater biomass than *R. maritima*, and a deeper depth limit than *R. maritima*, even in areas where they co-occur (Orth and Moore 1988). However, distributions of *Z. marina* along the east coast of the US are close to the southern limit for *Z. marina* water temperature tolerance, therefore *Z. marina* in this area is currently threatened by warming temperatures (Moore et al. 2012, Moore and Jarvis 2008). In contrast, *R. maritima* is potentially more tolerant of these changes, as it grows well in many southern regions of the U.S. coast (Kantrud 1991).

The long-term temperature rise in the Chesapeake Bay, recently estimated to be 0.8-1.1°C warmer than the 1950s (Preston 2004), is compounded by episodic warming events, defined as increased frequency and duration of high (>30 ^{o}C) summer water temperatures (Moore and Jarvis 2008). High temperatures can cause *Z. marina* to produce fewer shoots (Williams 2001), allow light-attenuating epiphytic algae to thrive (Brush and Nixon 2002) and increases hypoxic sediment stress to seagrass (Homer and Bondgaard 2001). *Z. marina* has experienced significant losses in the Chesapeake Bay; at present, populations are only 65% of what they were several decades ago (Moore et al. 2012, Orth et al. 2010). Several large-scale *Z. marina* die-off events have taken place in the Chesapeake Bay (Moore et al. 2014), and

increased prevalence of these events could result in eventual elimination of *Z. marina* from these systems (Moore et al. 2012, Moore and Jarvis 2008).

It is possible that *R. maritima* may expand into areas where *Z. marina* is declining in the Chesapeake Bay (Moore et al. 2014). *R. maritima* has been documented in many instances as becoming more abundant when other co-existing seagrass species decline (Cho and Poirrier 2005, Bologna et al. 2007, Johnson et al. 2003, Lopez-Calderon et al. 2010, Cho et al. 2009). In sites in the Chesapeake Bay where the two species co-exist along a shoreline gradient, *R. maritima* was found to have lower biomass in zones where it was mixed with *Z. marina* than in monotypic stands, suggesting the two are competing and that *Z. marina* has the competitive advantage (Wetzel and Penhale 1983). Co-existing seagrass species compete for resources such as light, nutrients and space (Micheli et al. 1998, McCreary 1991, Orth and Moore 1988), and when one species' population declines, the other could be released from competition.

It is important to note that there are morphological and physiological differences between *Z. marina* and *R. maritima* that could restrict *R. maritima* from colonizing areas formerly occupied by *Z. marina*. *R. maritima* is well-adapted to high-light environments (Wetzel and Penhale 1983), and along a shoreline gradient, it typically occurs in monotypic stands in shallow intertidal areas, in mixed stands with *Z. marina* in intermediate depth areas in patches or as an understory to *Z. marina*, before declining in abundance in subtidal, *Z. marina* dominated areas (Orth and Moore 1988, personal observations). *R. maritima* may also be restricted to lower wave environments than *Z. marina* (Orth and Moore 1988), since its roots are thin and only penetrate about 5 cm into the sediment (Kantrud 1991) while the *Z. marina* root structure is more robust and penetrates about 10 cm into the sediment (McRoy et al.

1972). However, *R. maritima* may have a competitive advantage in the Chesapeake Bay during the summer, when water temperatures frequently exceed 25°C, which is stressful to *Z. marina* growth (Zimmerman 1989), but not for *R. maritima* (Wetzel and Penhale 1983).

R.maritima also has a different life history strategy than *Z. marina*. It can colonize a given area quickly due to its rapid reproduction both sexually and asexually (Johnson et al 2003, Cho et al. 2009). A study in Chesapeake Bay showed that sites recently vegetated with *R. maritima* showed higher seed production compared to sites that were already vegetated with *R. maritima* (Silberhorn et al. 1996), indicating its colonizing proficiency. Additionally, *R. maritima* seeds have tough seed coats and can persist in the sediment for up to three years (Kantrud 1991), which is much longer than *Z. marina* seeds, which decrease in viability just 6 months after they are released (Jarvis et al. 2014). Despite these recruitment techniques, *R. maritima* beds can often be ephemeral and their abundance difficult to predict from year to year in the Chesapeake Bay (Moore et al. 2000, Orth et al. 2014).

Ecosystem Services

Sediment Stabilization and Erodibility

Seagrasses baffle wave energy and facilitate sediment deposition, causing a positive feedback loop in which water overlying seagrass beds is clearer, which facilitates seagrass growth (van derHeide et al. 2007). Shoot density, distance to the edge of the bed, and seagrass canopy height are factors that contribute to the drag effect seagrass creates on the water column (Hansen and Reidenbach 2013). The reduction in current velocity creates decreased sediment carrying capacity of overlying water, causing sediment particles to be deposited on

the seagrass bed sediment surface (Bos et al. 2007). Seagrasses also influence sediment resuspension (Ward et al. 1984). Benthic microalgae found on the surface of seagrass-vegetated sediments serve to increase sediment cohesion and decrease erosion potential (McGlathery et al. 2007). For these reasons, more fine material is usually found inside a seagrass bed than in adjacent unvegetated areas (Ward et al. 1984, Bos et al. 2007, Hansen and Reidenbach 2013).

Moore (2004) established that biomass is important when considering seagrass-induced sediment deposition, and found that approximately 25-50% of maximum biomass or 100 g dw/m^2 (dry weight) in the lower Chesapeake Bay was the threshold above which *Z. marina* caused a reduction in suspended sediment concentration of water overlying *Z. marina* beds. Bos et al. (2007) demonstrated that shoot density was also important when they showed that dense *Z. marina* accreted significantly more sediment than less dense *Z. marina.* Additionally, wave attenuation is generally greater both when the seagrass canopy occupies more of the water column (Fonseca and Cahalan 1992) and during flowering season, when flowering shoots exceed the height of vegetative shoots (Chen et al. 2007). Because *Z. marina* has a taller canopy than *R. maritima* and generally has more biomass throughout the growing season in the lower Chesapeake Bay (Wetzel and Penhale 1983; Moore et al. 2000), *Z. marina* may more effectively facilitate sediment particles to settle out of the water column.

Shear stress, caused by currents and wave action, is exerted on the surface of sediment as water flows over it, and is responsible for erosion and sediment resuspension (Sandford and Maa, 2001). Sediment characteristics such as particle size, cohesiveness, and biological factors influence the ability of shear stress to erode sediment (Sandford and Maa, 2001), and in the

case of a seagrass bed, shear stress could also be influenced by the shoot density and biomass of the seagrass as well as proximity to edges of the bed (Hansen and Reidenbach 2013, Luhar et al. 2008). Because seagrass canopies facilitate the settling out of suspended solids, when subjected to shear stress, sediments from seagrass beds may exhibit high sediment resuspension, which would be an indication of recent sediment deposition (Dickhut et al. 2009). *Z. marina* beds may be better able to facilitate sediment particles settling out of the water column because they have taller canopy heights than *R. maritima* (Wetzel and Penhale 1983); and because eroded material is indicative of recently deposited material, *Z. marina* sediments may be more erodible.

Epifaunal Abundance and Richness

The structure provided by both roots and above ground material of seagrasses support a greater abundance and richness of organisms than adjacent unvegetated areas (Edgar 1990, Orth 1984). This is a reflection of proximity to food resources, protection from predation, and protection from physical forces such as reduction of wave energy (Edgar 1990, Lewis 1984). However, abundance and richness of fauna can also depend upon the habitat complexity of seagrass; for instance, canopy characteristics such as shoot density and height, blade surface area, and biomass (Virnstien and Howard 1987, Stoner 1980, Orth et al. 1984). Because *Z. marina* and *R. maritima*, as well as other seagrass species which co-occur, have differing habitat complexity characteristics, it is possible that faunal abundance and richness will differ between stands of different seagrass species, even those growing in the same area.

In the Chesapeake Bay, seagrasses are home to epifauna (small animals that live in seagrass canopies) such as small gastropods, small arthropods like ispods, amphipods and crabs, and small fishes (Orth et al. 1984). Epifauna are an important part of the seagrass ecosystems in the Chesapeake Bay because they provide food for higher trophic levels and also feed on epiphytic algae which can overgrow seagrass and cause it to die back (Neckles et al. 1993). Many studies find that seagrass biomass is the most important factor in explaining abundance and richness of epifaunal communities (Attril et al. 2000, Lewis 1984, Stoner 1982). However, some studies have found that habitat complexity characteristics like shoot density, canopy height, and surface area are unique to specific seagrass species; and therefore that epifaunal communities can change based upon specific seagrass species, even within stands of different species that co-occur (Micheli et al. 2008, Moore 2011).

Because *Z. marina* has greater biomass than *R. maritima* (Wetzel and Penhale 1983; Moore et al. 2000), it is possible that more abundant and diverse epifaunal communities will be found in seagrass beds dominated by *Z. marina* rather than *R maritima* in the Chesapeake Bay. However, when *Z. marina* abundance seasonally decreases at the end of the summer and *R. maritima* biomass increases (Orth and Moore 1988), epifauna may become more abundant and diverse in seagrass beds dominated by *R. maritima*. For instance, Pardiek et al. (1999) found *R. maritima* to be a more important habitat than *Z. marina* for late-stage blue crab larvae in the York River, Chesapeake Bay, after *Z. marina* died back during a heat wave. Habitat complexity characteristics such as shoot density may play a role in this relationship as well; for instance, pinfish have been shown to forage more efficiently for their epifaunal prey in *R. maritima* than

in *Z. marina* (Moore 2011), and similar relationships may play a role in epifaunal community differences between the two species.

Objectives and Hypotheses

Given the potential for continued decline of the dominant seagrass species *Z. marina* in the Chesapeake Bay (Moore et al. 2014), an assessment of ecosystem services *Z. marina* provides is necessary in order to determine whether restoration or mitigation of lost ecosystem services will be needed. Additionally, an assessment of the ecosystem services provided by the species that may replace *Z. marina* in some areas, *R. maritima*, is also valuable. It is possible that in the polyhaline Chesapeake Bay, the opportunistic seagrass species, *R. maritima* may ameliorate some of the detrimental effects of the loss of the historically dominant species, *Z. marina*. The objective of this project is to evaluate quality of ecosystem characteristics and services of both *R. maritima* and *Z. marina* in the Chesapeake Bay by testing the hypotheses below:

1. *Z. marina* shoot biomass will exceed that of *R. maritima* in *Z. marina* dominated and mixed species stands, however the biomass and density of *Z. marina* will decrease in these areas from early to late summer while the biomass and density of *R. maritima* will increase.
2. Epifaunal abundance and richness will be greater in *Z. marina* and mixed stands than in *R. maritima*. However, this relationship may change if *Z. marina* drastically declines in biomass by the end of the summer.

3. Sediments will be finer in *Z. marina* stands than *R. maritima* stands. In late summer, however, this relationship may change as *Z. marina* dies back and becomes less dense.
4. Sediments from *Z marina* beds will resuspend more readily than sediment from *R. maritima* beds when subjected to shear stress.

METHODS

Site and Habitat Type Selection

Three seagrass sites in the lower Chesapeake Bay were chosen in 2013 for this study. The sites met two criteria; first, distinct areas vegetated with monotypic and mixed stands of *R. maritima* and *Z. marina* were present at similar depths (± 10 cm) and proximities to one another (between 50 – 300 m). Second, at each site chosen, previous monitoring had shown that *R. maritima* abundance had increased while *Z. marina* abundance had decreased in recent years. The Goodwin site (37.2188 Lat.,-76.4027 Long.) is located within the Chesapeake Bay National Estuarine Research Reserve in Virginia and has been monitored annually using underwater transects from 2004 to the present (Moore et al. 2014), while the Poquoson and Mobjack sites have been monitored annually by Virginia Institute of Marine Science personnel from 2008 to the present (Orth et al., unpublished data).

The three sites chosen represented a diversity of physical environments where the two seagrass species can be found in the lower Chesapeake Bay. The Mobjack site (37.3673 Lat., -76.4273 Long.) was located in an embayment surrounded by salt marsh, while the other two sites, Goodwin and Poquoson (37.1375 Lat.,-76.3260 Long.), were seagrass beds fringing saltmarsh habitats with greater exposure to the east and south respectively (Figure 1). While the sites were physically diverse, integrated monthly water quality monitoring around the three sites has shown similar levels of salinity, turbidity, and pH. These data were acquired from the Virginia Estuarine and Coastal Observing system (VECOS) website at http://www2.vims.edu/vecos.

Within each of the three sites, three distinct locations, or 'habitat types' were chosen: monotypic *Z. marina,* monotypic *R. maritima,* or 'mixed' stands where both species occurred. Within each of these habitat types, five replicate sampling areas were chosen by haphazardly throwing a meter-square quadrat within 10m of a pole marking the approximate center of each of the three stands. All samples were taken within each meter-square quadrat.

Biomass, sediment characterization and epifaunal samples were taken in June of 2013; the typical period with greatest *Z. marina* biomass in the Chesapeake Bay, and August of 2013, the period of *R. marina* greatest biomass (Moore et al. 2000). Sediment erodibility samples were taken in September of 2013, June and August 2014, only at the Goodwin site. Additionally, HOBO temperature loggers (Onset Computer Corporation, Bourne, MA) were attached to a PVC pole and placed in the 'mixed' areas of each site, where they were constantly submerged, beginning in May of 2013. The data loggers recorded temperature every 15 minutes through August of 2013.

Biomass Sampling

Biomass samples were obtained with a 12 cm diameter acrylic core, driven 10 cm into the sediment. Samples were sieved to remove sediment in the field and transported back on ice to the lab where they were separated into species, then separated into above and belowground portions, shoots were counted and dried in a 65°C drying oven until they reached a constant weight, then weighed.

Sediment Characterization

Sediment samples were obtained with a 7cm diameter acrylic core driven 15 cm into the sediment and transported back to the lab on ice, where they were sectioned into 0-2, 2-5, and

5-10cm depth fractions. Each core section was divided into portions used for organic matter, pore water nutrient, and grain size measurements. Sediment grain size was analyzed by separating sand from the sample and then using a pipetting method to determine silt and clay fractions (modification of Plumb, 1981). Silt and clay fractions were combined for a measurement of overall fine sediment compared to sand.

Epifauna Sampling

Epifaunal samples were collected using a Virnstein grab (Virnstein and Howard 1987). The grab was closed onto the seagrass canopy, shoots were trimmed at the sediment-water interface, put in a mesh bag, and frozen in the lab until being processed. During processing, epifauna was separated from seagrass shoots, and placed into a 70% ethanol solution until being identified. Seagrass shoots were dried in a 65°C drying oven until samples reached a constant weight. Epifauna were sieved using a 0.5mm mesh and identified to species, except in very few cases when they were identified to genus level.

Sediment Erodibility

Erodibility measurements were determined using a Gust Erosion Microcosm (Green Eyes, Cambridge, MD) which uses a magnetic spinning plate to create shear stress on the surface of sediment in order to erode material (Dickhut et al. 2011, Gust and Muller 1997). For this sample type, only sediments from monospecific *Z. marina* and *R. maritima* stands were compared. Samples were taken using a 10 cm diameter core. Sampling occurred within an hour of high or low tide to minimize disturbance to the surface of the sediment. To avoid clogging the Gust with organic matter and shoots, cores were taken in small unvegetated areas within the *Z. marina* or *R. maritima* stands. Samples were taken by driving the core ~15 cm into the

surface of the sediment. A circular extruder matching the inside diameter of the core tube was used to gently push the sediment in the tube upward until its surface was 10 cm below the top of the tube. A cap was placed on top to seal in the overlying water, electrical tape was used to seal them shut, and samples were immediately transported in the dark back to the lab. Site water was also collected in carboys to use as replacement water during the course of the experiments. Experiments were begun using the Gust within an hour after the cores were returned to the lab, in order to minimize sediment consolidation. After an initial flushing period to reach stabilization, the cores were exposed to seven increasing levels of shear stress; 0.01, 0.05, 0.10, 0.20, 0.30, 0.45 and 0.56 Pa, each level ran for 10 minutes. Water was collected and filtered onto pre-combusted and weighed 0.7 μm Whatmann GF/F filters using vacuum filtration. Filters were dried and weighed, and TSS (total suspended solids) determined by weight difference. Filters were then combusted at 550°C and weighed again to determine inorganic suspended solids.

According to Ward et al. (1984) in the Choptank River, a northern Chesapeake Bay tributary, 25 km/hr winds generated 25 cm/s current velocities, which eroded a bare area adjacent to a seagrass bed. Lawson et al. (2012) carried out a Gust experiment in a coastal bay of the Eastern Shore, VA, where they assumed 0.32 N/ m^2 shear stress was roughly equivalent to a 25 cm/s current velocity, which they calculated to be in the range of shear stresses that would be generated during a storm in a shallow bare area. Using a model run previously (Gong et al. 2007), it was confirmed that 25 cm/s is a current speed that can also be observed during storms at the Goodwin site (Jian Shen personal communication), where cores for the Gust

experiment were taken. Therefore the bed stress of 0.30 Pa used in this experiment roughly corresponds to storm-generated shear stresses.

Data Analyses

Temperature data, which were recorded every 15 minutes, were integrated into a daily mean of 96 observations, and assumptions of ANOVA were tested. Data were homogeneous (Levene's test p=0.7156) but not normal (Anderson-Darling test p=0.01861). However, because ANOVA is robust to departures from normality, especially with large sample sizes (Underwood 1997), ANOVA was performed on the dataset.

There were many zeros in the biomass data that corresponded to a seasonal dieback of *Zostera marina* at these sites, which contributed to the data not meeting ANOVA assumptions. Transformations were applied and data still did not meet the assumptions. Therefore, an Align Rank Transform non-parametric test was performed (Wobbrock et al. 2011); a non-parametric test that can be used with more than two main effects. An interaction of the main effects was tested with the R package (R Core Team 2013) 'lsmeans', which uses Tukey's HSD (Honestly Significant Difference) tests for multiple comparisons.

Epifaunal data were evaluated by species richness and abundance. The N was three for all samples. Because the measurements of epifauna were count data, a GLM was used and a poisson distribution specified. Analysis of deviance was performed on the result of the GLM to determine significance. The amount of biomass in the sample, akin to available habitat, as well as shoot density, were used as an additive covariates in the GLM. The 'effects' package in R was used to incorporate the covariate 'biomass' into the predictors of 'month', 'site' and 'habitat' (Fox 2003).

Grain size data were tested between habitats within each month, and between habitats across the two months. Silt and clay fractions were combined in grain size measurements for a total value representing fine sediment. Only the endmembers, the *R. maritima* and *Z. marina* habitat types were evaluated. Data were log-transformed to meet assumptions of ANOVA in all three cases. After transformation, the dataset from June samples had both homogeneity of variances and was normal (Levene's test p=0.1935 and Anderson-Darling test 0.4186). The data from August was also both homogeneous and normal (Levene's test p=0.9929 and Anderson-Darling test p=0.2655). The entire dataset including both months had homogeneity of variances and was normal (Levene's test p=0.6908, Anderson-Darling test p=0.4893). Type III ANOVA was run in all three cases because of the loss of 9 (out of 108) samples. Post-hoc comparisons were made with Tukey's HSD, and interactions of the man effects were also tested with Tukey's HSD. N was 3 in most cases.

Sediment erodibility values were evaluated for the endmembers, the habitat types *R. maritima* and *Z. marina.* Eroded mass values from the *Ruppia* and *Zostera* habitat types were tested using a Student's T-test.

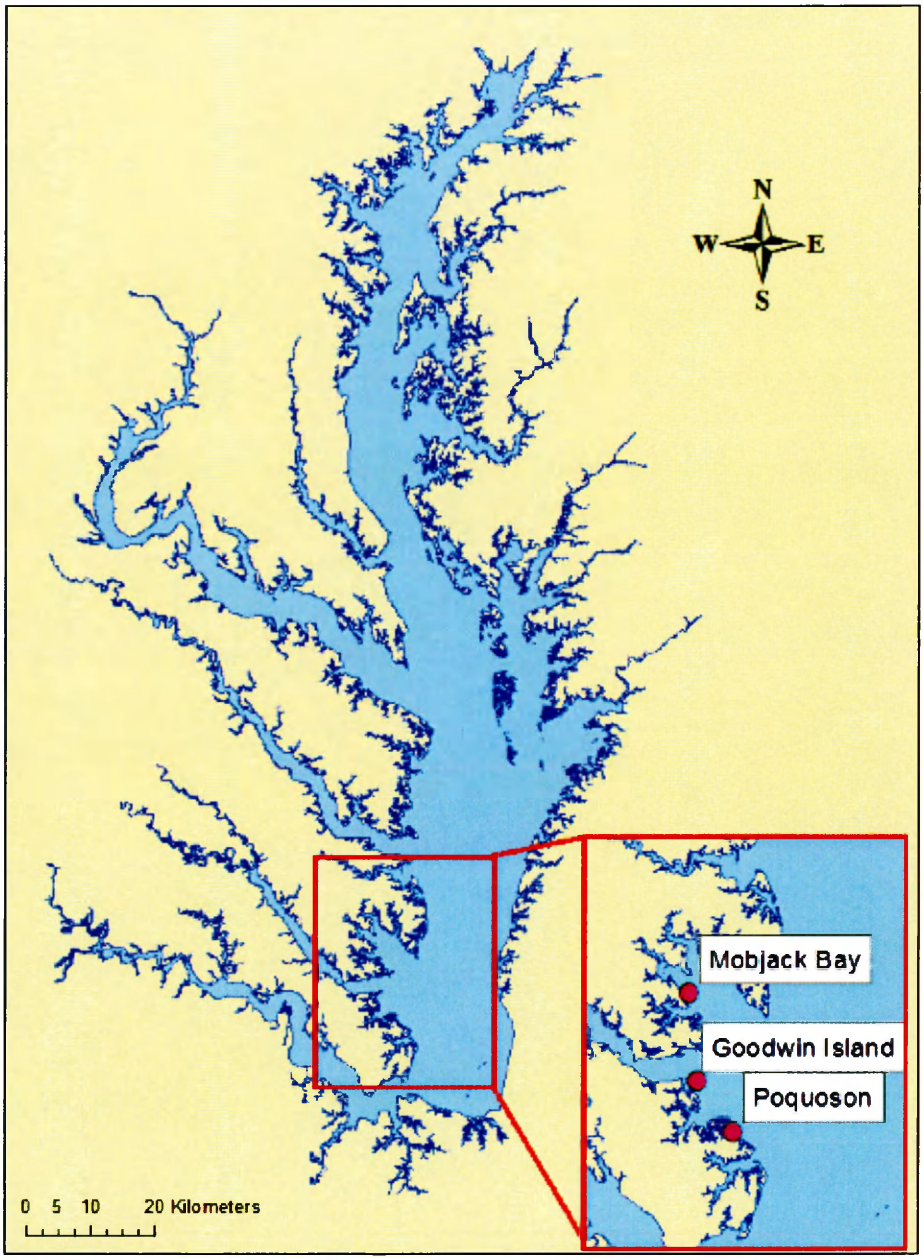

Figure 1. Study sites in the lower Chesapeake Bay.

RESULTS

Water Temperature

During the summer of 2013, mean daily water temperatures were highest in mid-July, and showed trends consistent with spring and neap tidal cycles. Water temperatures at the Mobjack site were significantly higher than both the Goodwin and Poquoson sites (p adj. <0.01 and <0.05 respectively). The Goodwin and Poquoson site summertime daily mean water temperature averages were not significantly different from each other.

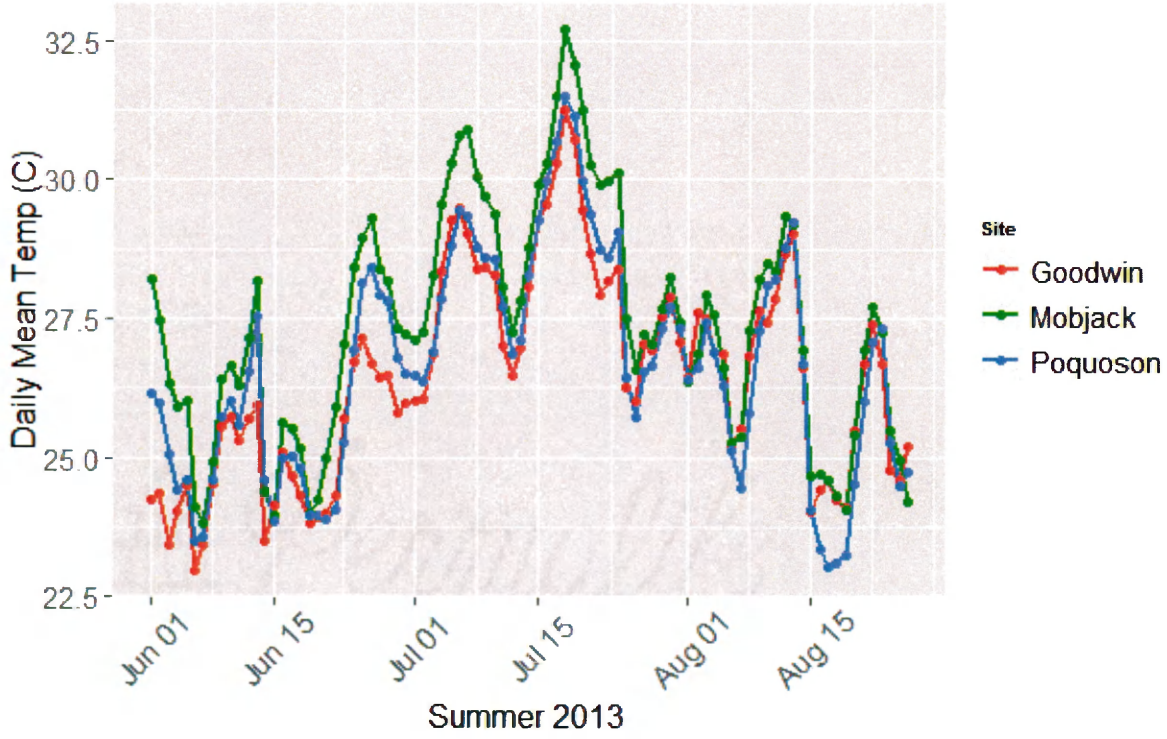

Figure 2. Water temperatures at three Chesapeake Bay sites from June 1st to August 30th of 2013.

Biomass and Shoot Density

Biomass samples represented the habitat type they were taken in, and the *R. maritima* and *Z. marina* habitat types only had minor amounts of *Z. marina* and *R. maritima* in them, respectively. If *R. maritima* was found in a *Z. marina* habitat type core or vice versa, the shoots were counted and biomass was recorded. In June, when sites and habitat types were established, shoot densities in biomass samples were an average of 88% *Z. marina* and 12% *R. maritima* across the *Z. marina* habitat types, 96% *R. maritima* and 4% *Z. marina* across the *R. maritima* habitat types, and 26% *R. maritima* and 74% *Z. marina* across 'mixed' habitat types.

Across all sites, biomass significantly declined from June to August of 2013 (Table 2a). Biomass declined across months and habitat types as well, although there was an interaction between the two. Analysis yielded that the three habitat types at Mobjack exhibited a significant decline, while the other habitat types exhibited a trend of decline (Figure 3). Across the sites, *Z. marina* habitats showed an 84% decline in biomass from June to August, while *R. maritima* habitats declined 49% and mixed areas declined 75% (Table 2b). Although in the Chesapeake Bay, *R. maritima* typically increases in biomass from the beginning to the end of the summer (Moore et al. 2000); there was a decline in *R. maritima* habitats types as well.

Across sites, shoot density in *Z. marina* habitat types fell from an average of 3331 to 1491 shoots/m^2. *R. maritima* habitat types also exhibited a trend of declining shoot density, from 9296 to 5936 shoots/m^2. Mixed area shoot densities fell from a mean of 8788 shoots in June to 1656 shoots m^2 in August (Table 3b).

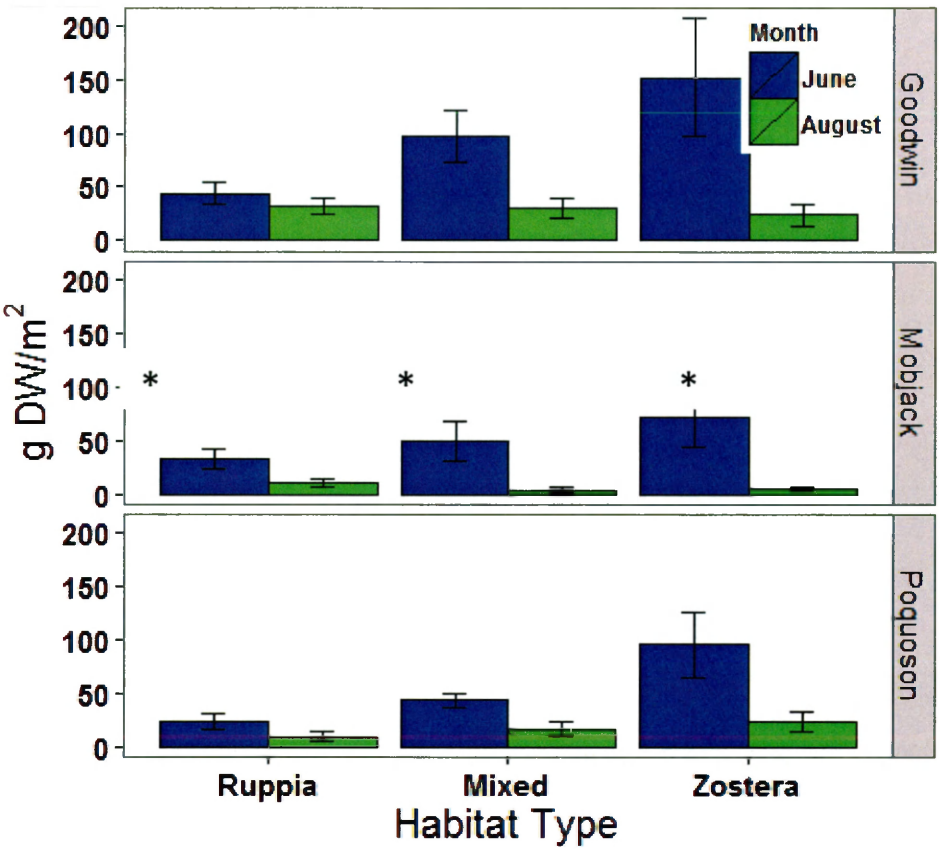

Figure 3. Above ground biomass means (n=5), p-value <0.05 indicated by *.

Effects	F-value	P-value
Month	47.3696	**1.223e-10**
Site	11.0525	**3.165e-5**
Habitat	8.3804	**0.0003**
Month: Site	1.9202	0.1499
Month: Habitat	9.0363	**0.0002**
Site: Habitat	1.1294	0.3445
Month: Site: Habitat	1.3837	0.2419

Table 1a. ANOVA table for above ground biomass (n=5).

Month	Site	Mean site biomass (g DW/ m^2)	Habitat type	Mean habitat type biomass (g DW/ m^2)
June	Goodwin	97.906	*R. maritima*	44.400
			Mixed	97.434
			Z. marina	151.884
	Mobjack	51.356	*R. maritima*	33.130
			Mixed	49.699
			Z. marina	71.240
	Poquoson	53.783	*R. maritima*	23.354
			Mixed	42.967
			Z. marina	95.028
August	Goodwin	28.951	*R. maritima*	32.337
			Mixed	30.591
			Z. marina	24.036
	Mobjack	6.142	*R. maritima*	9.943
			Mixed	3.425
			Z. marina	5.060
	Poquoson	16.522	*R. maritima*	9.519
			Mixed	16.534
			Z. marina	23.513

Table 1b. Mean above ground biomass collected over two months at three sites and three habitat types.

Month	Site	Habitat type	Mean habitat type shoot density (shoots/ m^2)
June	Goodwin	*R. maritima*	8877.335
		Mixed	4650.875
		Z. marina	2917.849
	Mobjack	*R. maritima*	8629.760
		Mixed	9036.491
		Z. marina	4898.450
	Poquoson	*R. maritima*	10380.470
		Mixed	12679.381
		Z. marina	2175.124
August	Goodwin	*R. maritima*	9903.004
		Mixed	1662.290
		Z. marina	1290.927
	Mobjack	*R. maritima*	3713.626
		Mixed	1556.186
		Z. marina	2033.653
	Poquoson	*R. maritima*	4191.093
		Mixed	1750.710
		Z. marina	1149.456

Table 2a. Mean shoot densities collected over two months at three sites and three habitat types.

Month	Habitat type	Mean habitat type shoot density (shoots/ m^2)
June	*R. maritima*	9295.855
	Mixed	8788.916
	Z. marina	3330.474
August	*R. maritima*	5935.908
	Mixed	1656.395
	Z. marina	1491.345

Table 2b. Mean seagrass shoot densities over two months from the three habitat types.

Grain Size and Erodibility

Across both months, the effect of habitat type on sediment grain size was significant ($p<0.0001$, Table 5), although there was also a significant interaction with sites ($p<0.05$). Analyses yielded that at Mobjack, *Z. marina* had greater fine sediment than *R. marina* across both months (p adj. <0.001). At Poquoson, *Z. marina* also had greater fines than *R. maritima* across both months (p adj. <0.001). At Goodwin, the *R. maritima* and *Z. marina* habitat types were not significantly different from each other. There was also an interaction between the effect of month and site, which highlighted the fact that Mobjack sediments became coarser from June to August. The effect of depth on percent fine sediments was significant across

months, sites and habitat types ($p<0.05$). The depth 0-2 cm had significantly greater fine sediments than the depth 2-5 cm (p adj. <0.05), and marginally greater fine sediments than the depth 5-10 (p adj. $=0.06$).

Within each month, there were also interesting relationships. In June, the effect of habitat type on sediment grain size was not significant, although mean percent fine sediment is greater in *Z. marina* sediment than *R. marina* sediment at Poquoson and Mobjack. In August, that relationship became significant; there was a greater amount of fine sediment in *Z. marina* habitat types at Poquoson and Mobjack ($p<0.001$ and $p<0.01$, respectively, Figure 4).

Overall, *Z. marina*- dominated beds had an average of 88% sand and 12% silt and clay, while *R. maritima* dominated beds had an average of 90.5% sand and 9.5% silt and clay. These measurements are comparable to observations by Moore (2004), who found inside a *Z. marina* bed, sand and gravel accounted for 87% of material while silt and clay accounted for 13%, and outside the bed 94% of material was sand and gravel while 6% was silt and clay.

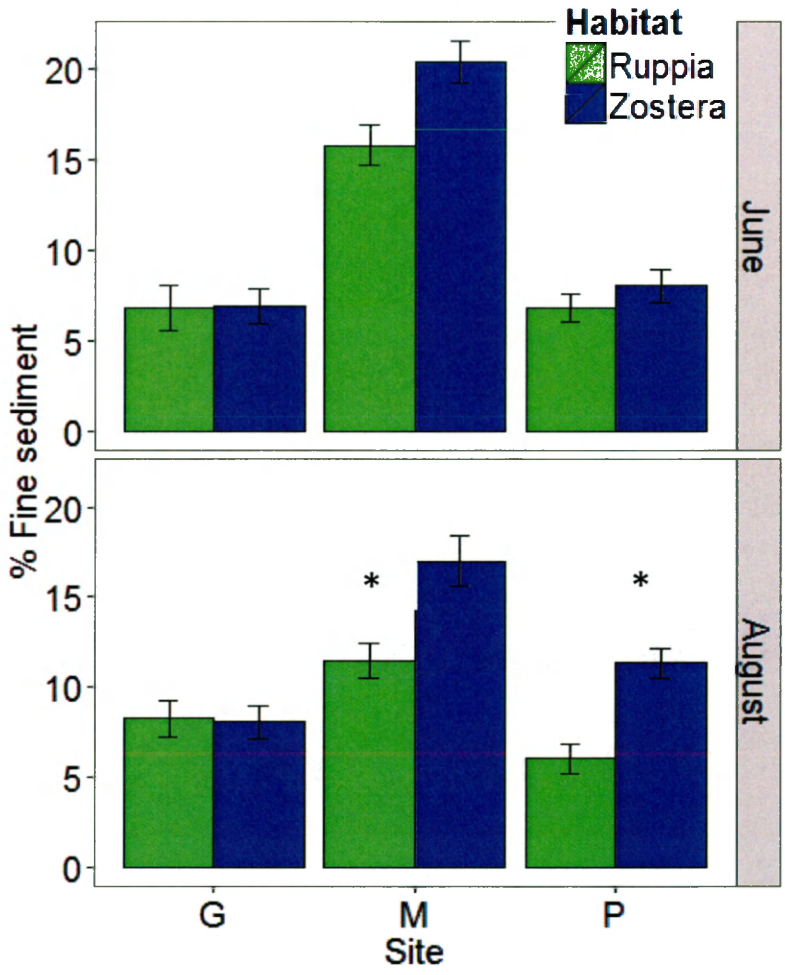

Figure 4. Sediment grain size- percent fine sediment, p-value <0.05 indicated by *. In August at Poquoson and Mobjack, the *Z. marina* habitat type had more fine sediment than the *R. maritima* habitat type. Not shown: there were also more fine sediment in the *Z. marina* habitat type than the *R. maritima* habitat type over both months at Poquoson and Mobjack.

Effects	F-value	P-value
Month	0.0008	0.977378
Site	70.6240	**<2.2e-16**
Habitat	19.0060	**4.911e-05**
Depth	5.3667	**0.007044**
Month: Site	5.2311	**0.007911**
Month: Habitat	2.6568	0.108097
Site: Habitat	4.3219	**0.017423**
Month: Depth	0.7153	0.492950
Site: Depth	0.4462	0.774741
Habitat: Depth	1.2714	0.287539
Month: Site: Habitat	1.4753	0.236502
Month: Site: Depth	0.79942	0.533408
Month: Habitat: Depth	0.2616	0.770688
Site: Habitat: Depth	0.8432	0.503098
Month: Site: Habitat: Depth	1.7486	0.150506

Table 3. ANOVA table of sediment grain size.

Due to inadequate replication after the Gust Mesocosm did not operate properly at high stresses, the stresses 0.45 and 0.56 Pa were omitted in the analysis. Using the remaining stresses, in June of 2014, significantly more (p=0.01) sediment was eroded from the cores representative of the *Z. marina* habitat type than the cores representative of the *R. maritima* habitat type. In August, the opposite relationship is demonstrated; significantly more (p=0.005) sediment was eroded from the cores representative of the *R. maritima* habitat type that the cores representative of the *Z. marina* habitat type (Figure 5). Interestingly, over the five stresses, a similar amount of mass was eroded from *R. maritima* in August than from *Z. marina* in June, and these masses eroded were not significantly different.

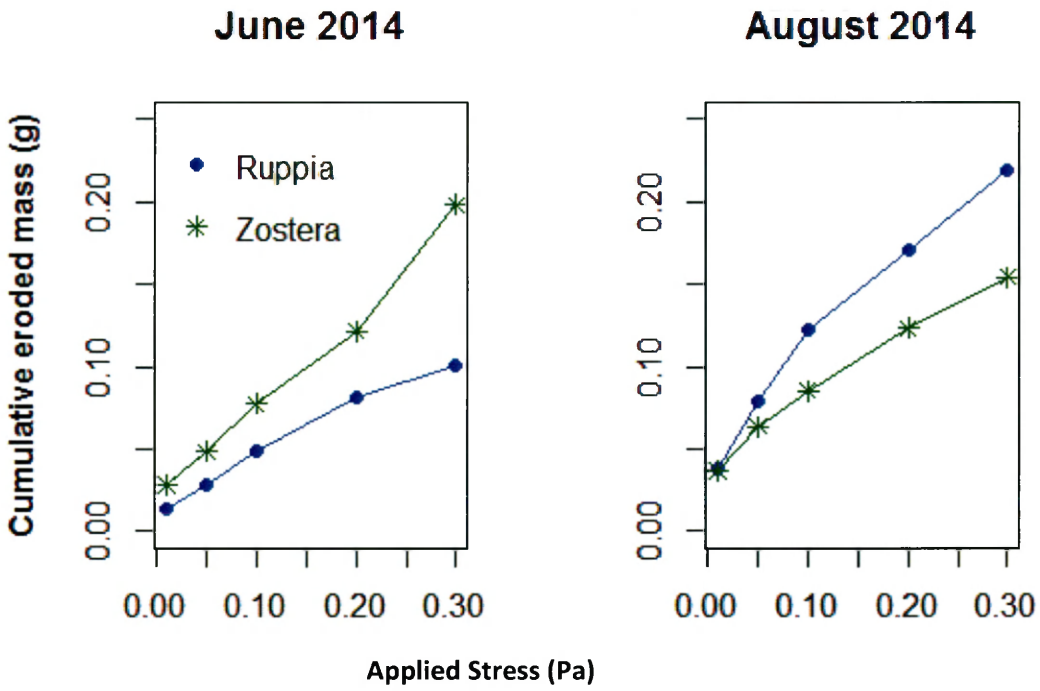

Figure 5. Sediment erodibility. Eroded mass is significantly different between habitat types within both months.

Epifaunal Abundance and Richness

Epifaunal species richness was not different between habitat types across or within months. The only significant effect on epifaunal species richness was that of month; from June to August, richness decreased across sites and habitat types (Table 4a). Actual epifaunal richness fell from a mean of 5.96 (±0.29 SE) individuals in June to 2.93 (±0.27 SE) individuals in August.

Effects	LR Chi-square	P-value
Month	10.0247	**0.001545**
Site	0.2286	0.8919
Habitat	1.5892	0.4518
Seagrass Biomass	0.0432	0.8354
Macroalgal Biomass	0.5821	0.4455
Shoot Density	0.1420	0.7063
Month: Site	1.7996	0.4067
Month: Habitat	0.8902	0.6408
Site: Habitat	3.1269	0.5368
Month: Site: Habitat	0.3816	0.9840

Table 4a- Analysis of deviance- epifaunal richness (n=3).

Across months, epifaunal abundance was significantly affected by habitat type; however there were interactions of habitat with site and month (Table 4b). Multiple comparisons revealed that there were no consistent effects of habitat type over the sites and months. Within June, at Mobjack and Poquoson, there was significantly greater abundance of epifauna in *Z. marina* than *R. maritima*. Also within June, at Goodwin and Poquoson, there was significantly greater epifaunal abundance in the 'mixed' habitat type than the *R. maritima.* Within August, there were significant relationships; however none were consistent. At Goodwin, *Ruppia* had significantly more epifauna than the 'mixed' or *Z marina* habitat types. At Mobjack, the only significant relationship was that the *Z. marina* habitat had more epifauna than the mixed, and at Poquoson, the 'mixed' habitat type had more abundant epifauna than both the *R. maritima* and *Z. marina* areas (Figure 6).

Effects	LR Chi-square	P-value
Month	1790.73	**2.2e-16**
Site	826.69	**2.2e-16**
Habitat	287.03	**2.2e-16**
Seagrass Biomass	62.92	**2.148e-15**
Macroalgal Biomass	84.73	**2.2e-16**
Shoot Density	38.24	**6.244e-10**
Month: Site	5.97	0.05054
Month: Habitat	78.82	**2.2e-16**
Site: Habitat	390.14	**2.2e-16**
Month: Site: Habitat	238.92	**2.2e-16**

Table 4b- Analysis of deviance- epifaunal abundance (n=3).

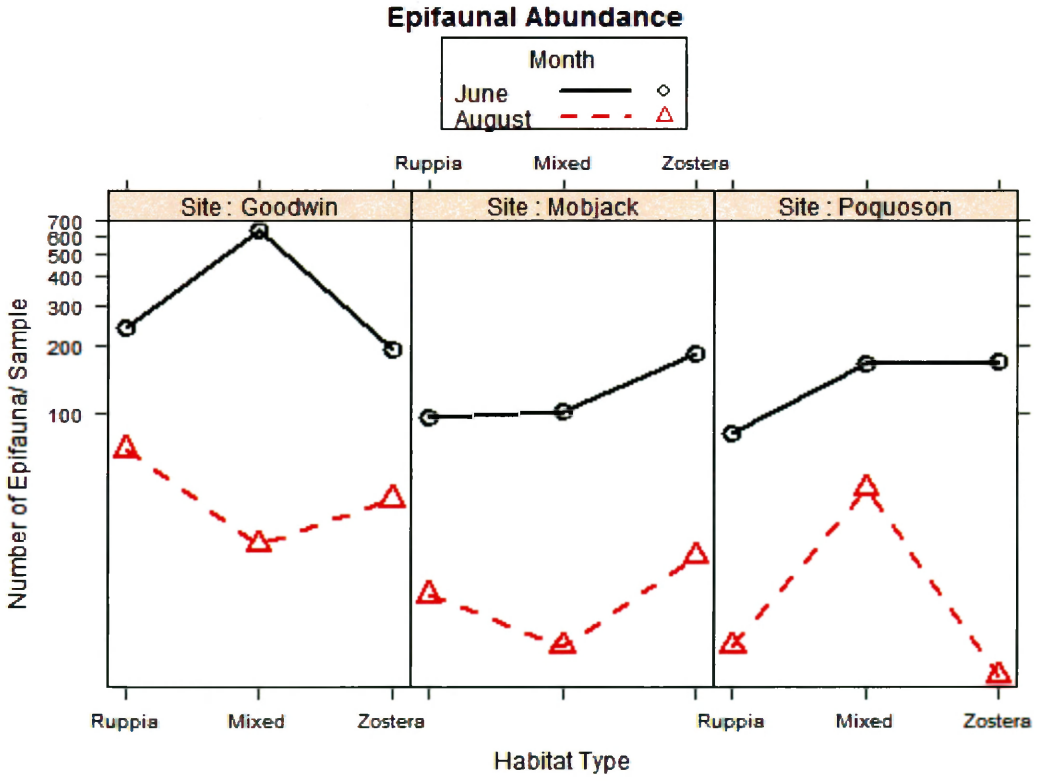

Figure 6. Epifaunal abundance across sites, months and habitat types (n=3)

DISCUSSION

Typically in the southern Chesapeake Bay, *Z. marina* decreases in abundance toward the middle to late summer while *R. maritima* increases in abundance around the same time (Wetzel and Penhale 1983, Orth and Moore 1988). However in this study, *R. maritima* biomass declined from June to August of 2013. While this is atypical of *R. maritima* in this region (Wetzel and Penhale 1983, Orth and Moore 1988), observations suggest that adjacent areas to where sampling occurred showed qualitatively more *R. maritima* over the course of the summer. Shoot density in the *R. maritima* habitat type at one site increased between June and August, while at the other two sites it fell. The inconsistencies of *R.maritima* biomass increasing outside the original sampling areas while decreasing inside them points to the fact that *R. maritima* can have unpredictable growth trajectories during the summertime growing season and its populations may be less stable and more ephemeral than those of *Z. marina*. Therefore the ecosystem services and functions associated with this species may likewise be more ephemeral.

'Mixed' habitat types did not become *R. maritima*-dominated over the course of the summer as *Z. marina* died back. Though there are many sources that have found *R. maritima* or a similar small, quickly-colonizing species can replace *Z. marina* when environmental conditions are not favorable for *Z. marina*; the switch can take multiple seasons to occur. For example, Moore et al. (2014) found that after a major *Z. marina* dieoff event in the York River, VA, USA, during the summer of 2010, it wasn't until the following summer that *R. maritima* was able to colonize the area. Similarly, *Halodule wrightii*, another seagrass characterized as an early successional colonizer, replaced *Thalassia testidinum,* a long-lived, established seagrass species, in areas where *T. testudinim* was formerly dominant in Florida; but it took between 1-4 years

(Fourqurean et al. 1995). Although *R. maritima* in the Chesapeake Bay and elsewhere can colonize new areas through widespread sexual reproduction (Silberhorn et al. 1996, Cho et al. 2009), this process may take several growing seasons.

Sediments were finer overall in *Z. marina* habitat types than *R. maritima* habitat types at two sites out of the three; suggesting that *Z. marina* typically possesses a more enhanced capability to trap sediment than *R. maritima*. Seagrasses in general serve to reduce flow velocity inside the canopy, but sediment trapping capabilities vary with height as well as shoot density and morphology of the plant (Hansen and Reidenbach 2013), and *Z. marina* and *R. maritima* have differing characteristics. The finding that *Z. marina* sediments were finer at two sites agrees with the fact that waves are attenuated more effectively when shoots occupy more of the water column (Fonseca and Cahalan 1992), because *Z. marina* has a taller canopy that *R. maritima*. Fine sediment percentages were similar and not statistically different between habitat types at the third site, Goodwin, site over the summer.

The Mobjack site proved to be an interesting case study in seasonal sediment accumulation and release. Mobjack had significantly higher water temperatures than the two other sites during the course of the summer, and was the only site to show a significant decrease in plant biomass in both of the habitat types. In both habitat types, sediment coarsened from June to August, a process referred to as sandification (Katwijk et al. 2010). In August, almost all plant material had detached and plants were floating in rafts above the sediment. Detached plants were not flushed out of the site presumably because the site was an embayment of Mobjack Bay, already an embayment itself of the York River; with low wave

action and tidal energy relative to the other two sites in this study. It is likely that the high temperatures caused this almost complete loss of biomass, which caused formerly consolidated fine sediments to be resuspended. The phenomenon of sandification is not unprecedented; sediment accumulated during the growing season by seagrass can dissipate after plants senesce in the fall (Bos et al. 2007), but it probably occurred earlier in the summer at this site than other sites.

Sediment erodibility measurements from the Goodwin site during summer of 2014 showed that *Z. marina* sediment was more erodible in June, while *R. maritima* sediment was more erodible in August. The June results agree with the grain size data from summer 2013 which suggested *Z. marina* was more efficient at trapping fine sediment than *R. maritima*. The August results, though unexpected, make sense when the 2013 shoot density and biomass data were examined. At the Goodwin site from June to August, *Z. marina* decreased in shoot density, while *R. maritima* increased, and both species had similar amounts of biomass by August; although *R. maritima* had greater mean biomass. Although biomass and shoot density data were not taken for 2014, if they are similar to the 2013 data, then the heightened shoot density between June and August and the greater biomass of *R. maritima* than *Z. marina* in August may be the reasons why *R. maritima* sediment were more erodible than *Z. marina* sediments in 2014.

In previous Chesapeake Bay studies, both seagrasses have been shown to be effective at trapping fine suspended sediment (Moore 2004, Ward et al. 1984). The differing biomass of the two species, however, is likely to be the ultimate factor in the difference between their abilities

to trap sediment. In June, though mean shoot density in the *R. maritima* habitat types was higher than that of the *Z. marina* habitat types at all three sites, the *Z. marina* habitat types across sites still had greater biomass, and mean percent fine sediment was higher in *Z. marina* than *R. maritima* habitat types at two of the three sites, Mobjack and Poquoson. In August, this trend became statistically significant; *Z. marina* habitat types at Mobjack and Poquoson had accumulated more sediment than *R. maritima* habitat types. Although the August 2014 sediment erodibility data actually showed that *R. maritima* areas trapped more sediment, it is important to keep in mind that this measurement likely reflects shorter-term deposition events than the sediment grain size measurements from this study. In a similar seagrass sediment erodibility study using the Gust mesocosm, only the top 3 mm of sediment was eroded (Lawson et al. 2012), while with the sediment grain size data, 10 cm of sediment was being evaluated.

After accounting for the differences in shoot density and biomass of the two seagrasses, it was shown that at two sites during June, *Z. marina* habitat types had significantly greater epifaunal abundance than *R. maritima* habitat types, suggesting that during the growing season for *Z. marina*, it provides a better quality habitat for epifauna that *R. maritima*. Additionally at two sites during June, there were significantly greater epifaunal abundances in the 'mixed' habitat than the *R. maritima*. During June, 'mixed' habitat types had greater shoot densities of *Z. marina* than *R. maritima*; because in areas where the two are mixed, *Z. marina* usually has the competitive advantage (Wetzel and Penhale 1983). Higher epifaunal abundance in the 'mixed' habitat types could therefore indicate that the presence of *Z. marina* is enhancing the quality of the habitat in the mixed areas for epifauna.

In August, the relationships demonstrated in June were no longer present, and relationships in all the habitat types were inconsistent. This is probably because the abundance of epifauna associated with seagrasses is closely tied to habitat availability (Orth and Heck 1980), and patchiness that is the result of seasonality-associated dieback can increase predation on epifauna (Moore and Hovel 2010). One interesting relationship that occured in August of 2013 is the fact that there were more epifauna in *R. maritima* than the other habitat types at one site, Goodwin. Goodwin was the only site to increase in *R. maritima* shoot density from June to August, and its biomass stayed stable between June and August, compared to the other sites, where it declined. This could suggest that at Goodwin, abundant *R. maritima* provided a better habitat for epifauna than *Z. marina* beds, which were dying back and actually had slightly lower mean biomass at the time.

Epifauna derive both protection from higher trophic levels and food from seagrass beds, and *Z. marina* and *R. maritima* provide these differently. There is more interstitial space in between shoots of *R. maritima* compared to *Z. marina*, which could potentially cause *Z. marina* to provide better refuge. Additionally, because epifauna use epiphytes as a food source, their abundances are closely tied to available epiphytes (Whalen et al. 2013). The strap-like blade of *Z. marina* could provide a better surface for the growth of epiphytic algae. Relative to both these points, the blades in each shoot of *R. maritima* are more tightly bundled that those of *Z. marina* (personal observation), which could provide less surface area for epiphyte growth and also less habitat complexity for associated epifauna.

Epifaunal abundance decreased significantly across all sites and habitat types over the summer, likely a result of the decrease in available habitat. Additionally, epifaunal richness was

not different between habitat types, but also fell across sites and habitat types from 6 species in June to 3 species in August. The lack of discernable trends between habitat types for epifaunal richness could be attributed to the fact that the lower Chesapeake Bay has a low richness of epifauna in general (Douglass et al. 2010).

During the height of the *Z. marina* growing season in June, *Z. marina* provided better quality of ecosystem services than *R. maritima*. In August, during the height of *R. maritima* growth, there is weak but present evidence that *R. maritima* can provide similar ecosystem services to *Z. marina*, albeit to a lesser degree, and more research is needed. *R. maritima* has a different seasonal maximum biomass than *Z. marina,* which may have major impacts for the ecosystem. Additionally, *R. maritima* populations are often ephemeral and it is hard to predict its distributions from year to year. Therefore, loss of *Z. marina* may be ameliorated to a degree by *R. maritima*, but the instability of R. maritima may make this capacity for ecosystem service replacement inconsistent. Finally, although *R. maritima* is recognized as having a higher temperature tolerance than *Z. marina*, it did not survive in Mobjack Bay, where temperatures reached 33 °C. This could have been a result of additive stressors, and *R. maritima* temperature tolerance in the Chesapeake Bay is a topic that needs more research.

Studying the relationship between *Z. marina* and *R. maritima* is not only of importance in the Chesapeake Bay at the southern limits of the *Z. marina* distribution on the east coast of the US, but on the west coast as well. Two studies, from San Diego Bay and Baja California (Johnson et al. 2003, Lopez-Calderon 2007), have detailed the rising abundance of *R. maritima* and simultaneous decline of *Z. marina*, and at the former, green turtles have incorporated *R. maritima* into their diet as it has increased in biomass. This study of ecosystem services

differences between *R. maritima* and *Z. marina* is likely applicable to other areas where the seagrasses coexist, as well as areas worldwide where dominant seagrasses are in decline and replacement by historically non-dominant species is occurring. The relationships between seagrass species and the ecosystems functions and services they provide are complex and warrant more investigation as climate and other factors including human influences in coastal areas continue to change.

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CHAPTER 2:

Sediment Microbial Communities in *Zostera marina* and *Ruppia maritima* Habitats

ABSTRACT

Seagrasses create heterogeneity and facilitate nutrient cycling in marine sediments by diffusing organic carbon and oxygen from their roots; rhizospheres are therefore hotspots for microbial activity. However, sediment microbial communities may differ according to vegetation characteristics. As the lower Chesapeake Bay is experiencing losses of *Zostera marina*, the seagrass species *Ruppia maritima* is replacing it in some areas. *R. maritima* is smaller than *Z. marina*, and has a less robust root system. The objectives of this study were to first examine whether species of seagrass affect microbial community assemblages, and next if microbial communities relate to the environmental variables sediment organic matter and pore water ammonium. Sediment microbial community composition, richness and diversity were determined by next generation sequencing of 16S rRNA genes using samples from *Z. marina* and *R. maritima* dominated sediments from two sites in the lower Chesapeake Bay during June and August 2013. During both months and at both sites evaluated, species of seagrass did not influence microbial community differences. Sediment organic matter was not correlated to microbial community composition, while sediment pore water ammonium showed a marginally significant correlation. The lack of differences among seagrass species observed suggests that the vegetation types had similar effects on the sediment microbial communities; however, more research is needed before determining whether microbial communities function similarly among these seagrass species.

INTRODUCTION

Seagrasses possess unique adaptations to living underwater, such as salinity tolerance and underwater pollination (den Hartog 1970). Another essential property seagrasses have is the ability to translocate oxygen down to the roots through a system of air lacunae (Roberts and Caperon 1986). Seagrasses occupy sediment in which bacterial reduction of sulfate is common, which produces sulfides that can be toxic to the plants (Jorgensen 1982). Seagrasses are able to overcome sulfide toxicity by translocating oxygen to their roots, re-oxidizing sulfides and rendering them no longer toxic (Roberts and Caperon 1986, Koch and Mendelssohn 1989). In addition to oxygen, dissolved organic carbon is also diffused out of the roots. The area where exudates escape and microbes interact with them around the roots is the rhizosphere (Brimcombe et al. 2000). These areas, as well as seagrass sediment in general, are areas of enhanced microbial activity and bacterial abundance (Lopez et al. 1995, Danovaro 1996).

The nutrient-cycling processes seagrasses use to sustain themselves also provide beneficial ecosystem services. Seagrasses have been referred to as a 'coastal filter' because of their ability to sequester and transform nutrient runoff; which has the potential to ameliorate eutrophication (McGlathery et al. 2007). Polychaetes, macroalgae, and seagrasses are all organisms that contribute to the heterogeneity of sediment and therefore facilitate nutrient cycling in shallow coastal areas (Kristensen and Blackburn 1987, Lawson et al. 2012, Caffery and Kemp 1990). Seagrass enhances denitrification, nitrogen removal from the system, by creating oxic- anoxic interfaces and releasing photosynthate into the sediment, which facilitates coupled nitrification-denitrification (Caffery and Kemp 1990, Shieh and Yang 1997, Eyre et al. 2011). Seagrasses are also comparatively longer-lived than other coastal primary producers, such as

phytoplankton and macroalgae, and can consequently retain nutrients for a longer period of time; improving water quality (Valiela et al. 1997, McGlathery et al. 2007).

Seagrasses both retain nutrients and facilitate sediment deposition; thereby causing a positive feedback loop of overlying water being clearer, which facilitates seagrass growth (van derHeide et al. 2007). If seagrass photosynthesis is impaired as a result of poor water quality or other environmental variables, however, translocation of oxygen to seagrass roots is interrupted, and toxic sulfides can accumulate. Greater inputs of organic matter can stimulate sulfate reduction (Holmer and Bondgaard 2001). Additionally, ammonium is produced when both allochthonous organic matter that becomes trapped in seagrass meadows and autochthonous material from plants dying back breaks down (Kemp et al. 1983, Pedersen et al. 1999). Seagrass roots take up ammonium for growth (Caffery and Kemp 1990), but too much ammonium in the water column could be toxic to seagrass (van Katwijk et al. 1997).

As referenced in Chapter 1, *Zostera marina* and *Ruppia maritima* are the two seagrasses that occupy the lower, polyhaline region of the Chesapeake Bay, USA. *Z. marina* growing here is close to its water temperature tolerance, and poor water quality and increasing frequency of high water temperature events are threatening its populations (Moore et al. 2012, Moore and Jarvis 2008). *R. maritima*, an efficient colonizer with higher water temperature tolerances than *Z. marina*, may expand into areas where *Z. marina* has declined; there are accounts of *R. maritima* encroaching on areas formerly occupied by *Z. marina* from not only the Chesapeake Bay, but New Jersey, USA, San Diego Bay, USA, and Northwest Mexico (Moore et al. 2014, Bologna et al. 2007, Johnson et al. 2003, Lopez-Calderon et al. 2010). In the lower Chesapeake

Bay, *Z. marina* reaches its greatest biomass in early summer, while *R. maritima* reaches its greatest biomass in late summer (Moore et al. 2000). Additionally, *Z. marina* has a more robust root structure than *R. maritima*; its roots penetrate 10 cm down into the sediment whereas *R. maritima* roots penetrate 5 cm into the sediment (Kantrud 1991, McRoy et al. 1972).

Seagrass rhizospheres are highly dynamic areas where marine sediments interface with plant roots that release oxygen and dissolved organic carbon; as a result, microbial activity is high in these areas (Kristensen et al. 2005). Electron microscopy has shown that rhizomes are the main site of colonization for morphologically diverse bacterial groups within seagrass sediments (Donnelly and Herbert 1999). Seagrass roots provide heterogeneity in the sediment, which introduces niches for different bacteria. For example, some sulfate- reducing bacteria are oxygen-tolerant, and therefore can colonize areas such as seagrass rhizospheres (Cifuentes et al. 2000); these bacteria may not be found in unvegetated sediments. Additionally, the presence of seagrass enhances coupled nitrification and denitrification (Caffery and Kemp 1990, Shieh and Yang 1997, Eyre et al. 2011), a microbially-mediated process that can utilize oxygen and carbon from seagrass roots. These factors are likely to be influenced by the differing rhizosphere characteristics of *Z. marina* and *R. maritima.* Since the root system of *R. maritima* is less robust and shallower than that of *Z. marina*, *Z. marina* sediment may have a more diverse and rich microbial community.

To test the possibility that microbial communities are more rich and diverse in *Z. marina* sediment compared to *R. maritima* sediment, next generation sequencing of 16S rRNA gene is used in this study. Next generation sequencing is a high-throughput technology that has

modified the examination of microbial communities based on a large number of 16S rRNA gene sequences (Roesch et al. 2007). Since 2008, many microbial sequencing studies have been published using this technology, which has made it possible to identify previously unknown taxa (Chariton et al. 2010). This high-volume approach facilitates the study of microbial community response to environmental factors (Hollister et al. 2010, Hudson 2008). Thus, this valuable tool was used to examine bacterial communities in seagrass sediments.

Objectives and Hypotheses

The objectives of this study are to compare richness, diversity and composition of bacterial communities in sediments that the two different seagrass species occupy. The data obtained are an important first step in evaluating the nutrient cycling potential in *Z. marina* versus *R. maritima* sediments; in order to make informed conservation and restoration decisions.

Hypothesis:

1) Microbial community diversity and richness will be higher in sediment occupied by *Z. marina* than sediment occupied by *R. maritima*.

METHODS

Sediment Microbial Community Analysis

Sediment samples were collected from two sites (Goodwin Island and Mobjack Bay) in the lower Chesapeake Bay during the summer of 2013 (Chapter 1, Figure 1). The Poquoson site studied in Chapter 1 was excluded because it shared similar physical environmental characteristics with the Goodwin site. The sampling plan was the same as that of the plan detailed in Chapter 1 of this thesis. The major difference was that only monotypic stands of the habitat types *Ruppia maritima* and *Zostera marina* were sampled; mixed stands were not. Samples were taken with a 7 cm acrylic core, sectioned into 0-2, 2-5 and 5-10 cm depth fractions in the field and placed on dry ice for transport back to the lab. Samples were kept in a -80°C freezer until they were ready to be processed.

Two replicate samples of sediment DNA were extracted using a PowerSoil DNA isolation kit (MO Bio Laboratory, CA, USA) following the manufacturer's protocol with some modification. A homogenized sediment sample (0.5 g) was used for DNA extraction. Quality and quantity of extracted DNA was examined with a NanoDrop spectrophotometer (DE, USA). Bacterial primers 27F and 338R, modified to include an 8 base pair barcode sequence, were used to amplify the V1 and V2 hypervariable regions of 16S rRNA genes (Arfken et al. 2015). Duplicate PCR reactions were conducted for each sample using Go-Taq master mix (Promega, Inc., WI, USA). The PCR cycle started with an initial denaturation step at 95°C for 3 min followed by 25 or 30 cycles of 95°C for 30 sec, 55°C for 1 min, and 72°C for 1 min and a final extension step at 72°C for 5 min. PCR products were pooled and purified using Wizard Gel and PCR Clean Up Kit (Promega, Inc., WI, USA). The concentration of purified products was measured on a

2200 TapeStation instrument using D1K reagents (Agilent Technologies, CA, USA). The amplicons were sequenced with the Ion Torrent 400 base pair sequencing kit (Life Technologies, Grand Island, NY) using the Ion Torrent PGM at the Virginia Institute of Marine Science.

Sequences were uploaded into the Ribosomal Database Project (RDP) Pipeline website (http://pyro.cme.msu.edu) for initial processing, and to trim and bin sequences in each library corresponding to a sediment sample. The program Acacia was used to de-noise and correct sequencing errors (Bragg et al. 2012). Sequences were then processed with PRINSEQ (Schmieder and Edward 2011) in order to change sequence header names and for size selection between 300 and 380 base pairs. The sequences were further analyzed using Mothur (version 1.4.1, Schloss et. al 2009). The sub.sample function was used to randomly select 10,000 sequences from each library to minimize the biases of different sequence numbers. Unique sequences were identified and aligned with the SILVA reference sequences. Precluster analysis and chimera check were conducted to obtain high quality sequences. Taxonomic classification of high quality sequences was determined based on the SILVA reference database. Operational taxonomic unit (OTU) was determined based on 97% sequence identity.

Abundances of bacterial families within each sample were analyzed with a 5% relative abundance cutoff in order to generate figures representing the most dominant bacteria within each sample. A cladogram of microbial community samples was obtained using the tree.shared function in Mothur, and rarefaction curve data were generated using the rarefaction.single function. Species richness data met the assumptions of ANOVA, and were tested using a type III, three-way ANOVA and t-tests. The Shannon and Inverse Simpson diversity indices for OTUs

were calculated using the collect.single function in Mothur. Data obtained met the assumptions of ANOVA, and were tested using a type III, three-way ANOVA and t-tests. Finally, Pearson correlation was run in R (R Core Team 2013) to test relationships between both sediment ammonium and organic matter, and diversity indices.

Sediment Organic Matter and Pore Water Nutrients

Sediment organic matter and pore water nutrient samples were taken from three sites. Sediment organic matter was determined via the loss-on-ignition method (Erftemeijer and Koch 2001) by drying samples in a 65°C oven, then combusting them at 500°C for 5 hours to determine the organic fraction. Sediment pore water nutrient concentrations were determined using Liao's (2001, revised 2002) methodology. Core portions were placed in a 2M KCl solution, shaken for one hour using a shake table, centrifuged for 6 minutes at 4000 RPM, filtered through 25mm syringe filters, and frozen for later analysis. When ready for analysis, samples were run on a Lachat auto analyzer (Loveland, CO, USA) for dissolved inorganic nitrogen.

Of the sediment pore water nutrients analyzed, only NH_{4+} was above detection limit, NO_2 and NO_3 were not. NH_{4+} data were not normal and were log-transformed to meet the assumptions of ANOVA. Data were then analyzed with a type III, four-way ANOVA, which yielded no interactions.

Organic matter data were log-transformed to meet assumptions of ANOVA. After transformation, data had homogeneity of variances, but was not normal (Levene's test $p=0.2491$, Anderson-Darling test $p=0.0003$). ANOVA was still preformed on all datasets, since ANOVA is robust to departures from normality, especially in large experiments (Underwood

1997). Type III ANOVA for unbalanced designs was run due to the loss of 3 (out of 120) samples. The N was five for most samples.

RESULTS

Microbial Community

After initial trimming in the RDP pipeline website, a total of 2,209,997 sequences were obtained (tables 1a and b). Microbial communities were not different among habitat types as hypothesized. Instead, communities showed the most differentiation in composition between months (Figure 1). Looking further into the differences between months, there is one family that is prevalent among the August samples (makes up over 5% relative abundance in 15 of 23 samples) but not among June samples (one of 24 samples), *Desulfobulbaceae*, which is capable of sulfate reduction (Kuever et al 2005). Additionally, again adhering to the 5% relative abundance within samples, June samples had three families that did not appear in August samples, but August samples did not have any families that did not appear already in June. Bacterial families that occurred consistently across months were *Flavobacteriaceae, Alteromonadaceae, Desulfobacteraceae*, and *Anaerolineaceae* (Figure 2), and there were 9 identifiable families that were over 5% relative abundance in all the samples combined.

Additionally, species richness and diversity were evaluated. There was no significant difference in both species richness and diversity in the different habitat types across or within months, or between months. However, Mobjack sediments had higher richness than Goodwin

sediments both within June and August and across months ($p<.001$ for June, $p<.01$ for August, $p<.001$ across months), (Figure 3). Diversity was also higher at Mobjack than Goodwin both within June and August and across months (within June: Shannon $p=0.001$, Inverse Simpson $p<0.005$, within August: Inverse Shannon $p<0.05$, Inverse Simpson $p<0.05$, across months: Shannon $p<0.001$, Inverse Simpson $p<0.001$).

June samples	Number of Sequences	Sequences after sub-sampling/ trimming	Coverage	#OTU	Inverse Simpson	Shannon	Chao	ACE
GR2-0-2	29896	9061	81.40%	2400	31.3	5.834	7503.3	13373.1
GR2-2-5	57174	9044	72.00%	3504	193	7.104	11422	22571.7
GR2-5-10	80684	9328	69.10%	4243	1071.2	7.784	11779.5	19214.2
GR3-0-2	57428	8877	72.40%	3358	184.5	6.988	11104.7	21714
GR3-2-5	45075	8848	70.90%	3563	353.9	7.27	11967.7	22542
GR3-5-10	117262	9144	69.80%	4063	877.7	7.675	10835.1	17121.5
GZ3-0-2	91512	9016	78.40%	2895	105.8	6.681	7895.7	13981.3
GZ3-2-5	41903	9406	75.30%	3463	480.7	7.303	9787	15420
GZ3-5-10	52355	9454	77.70%	3256	450.6	7.212	8610	13324.7
GZ4-0-2	121119	9004	76.50%	3225	229.8	7.111	8470.2	12791.4
GZ4-2-5	159592	9153	71.50%	3841	618.8	7.534	10272.3	17723.4
GZ4-5-10	66076	9296	70.10%	4003	717.3	7.606	11901.5	20086.3
MR3-0-2	27790	8650	66.00%	4017	718.9	7.607	13144	25877.7
MR3-2-5	45722	8655	57.50%	4787	942.2	7.906	16554.7	33743.1
MR3-5-10	21421	8771	53.80%	5255	1517.6	8.138	18674.4	34247.3
MR5-0-2	48927	8475	67.90%	3724	560.1	7.462	12108.6	24545.2
MR5-2-5	44446	9008	63.00%	4427	923.2	7.771	16808.8	32888.1
MR5-5-10	42577	9010	62.80%	4489	1104.7	7.826	15994.8	33088.8
MZ3-0-2	31682	8224	58.90%	4364	593.1	7.715	15930.6	33323.6
MZ3-2-5	45738	8356	56.70%	4598	703.7	7.788	18041.1	36675.1
MZ3-5-10	29611	8528	49.70%	5370	1266.9	8.146	20740.1	42706.7
MZ5-0-2	41930	8708	66.60%	3907	466	7.498	13916.2	28030.9
MZ5-2-5	43968	8768	59.50%	4624	968.7	7.86	17588.4	38727.2
MZ5-5-10	47077	9062	62.60%	4528	694.1	7.77	16182.9	29405

Table 1a. June Samples. Naming convention for samples includes, in order, the site (G for Goodwin, M for Mobjack), replicate number, and depth (0-2, 2-5, 5-10).

August Samples	Number of Sequences	Sequences after sub-sampling/ trimming	Coverage	#OTU	Inverse Simpson	Shannon	Chao	ACE
GR1-0-2	128305	8980	75.50%	3266	335.4	7.159	9005.3	15932.2
GR1-2-5	23027	9377	75.80%	3358	362	7.196	9608.8	16025.2
GR1-5-10	18724	9410	73.90%	3575	553.4	7.352	10630.3	18703.8
GR3-0-2	17308	9227	74.90%	3314	282.9	7.098	10368.7	19404.4
GR3-2-5	55615	9268	69.60%	4047	625.3	7.594	12038	19739.7
GR3-5-10	51018	9390	73.80%	3708	602.1	7.459	9529.2	16001.9
GZ2-0-2	63584	9113	78.30%	2985	182.6	6.842	7761.6	13153.9
GZ2-2-5	21989	9393	73.50%	3714	616.2	7.452	9981.8	16477.7
GZ2-5-10	11018	9278	66.80%	4191	919.9	7.677	15897.3	28453
GZ3-0-2	45265	9099	82.70%	2516	165.7	6.627	6355.7	9649.2
GZ3-2-5	30321	9303	76.20%	3309	378.4	7.209	9733.7	14961.8
GZ3-5-10	39473	9221	70.50%	3936	763.9	7.593	11133.7	20368
MR1-0-2	15672	9110	72.90%	3487	443.7	7.31	11994.4	20551.9
MR1-2-5	65612	9035	72.00%	3719	790.3	7.52	10213.4	17586.7
MR1-5-10	46900	9058	65.80%	4405	1468.1	7.885	13427.7	22199.8
MR2-0-2	15960	9032	76.40%	3114	424	7.121	9561.3	16325.7
MR2-5-10	11332	8988	70.50%	3777	808.4	7.539	11827.1	21631.9
MZ2-0-2	14462	9036	70.50%	3826	761.3	7.539	11175.4	20671.4
MZ1-2-5	37816	9092	71.20%	3788	747	7.537	11130.2	19750.1
MZ1-5-10	22877	8939	72.00%	3608	656.3	7.444	11016.7	18685.7
MZ3-0-2	25636	8968	73.20%	3408	461	7.265	10671.6	19500.3
MZ3-2-5	20444	9020	67.60%	4018	870.1	7.631	13860.4	26595.5
MZ3-5-10	36674	8983	65.60%	4385	1311.7	7.864	12861.9	22678.6

Table 1b. August Samples. Naming convention for samples includes, in order, the site (G for Goodwin, M for Mobjack), replicate number, and depth (0-2, 2-5, 5-10).

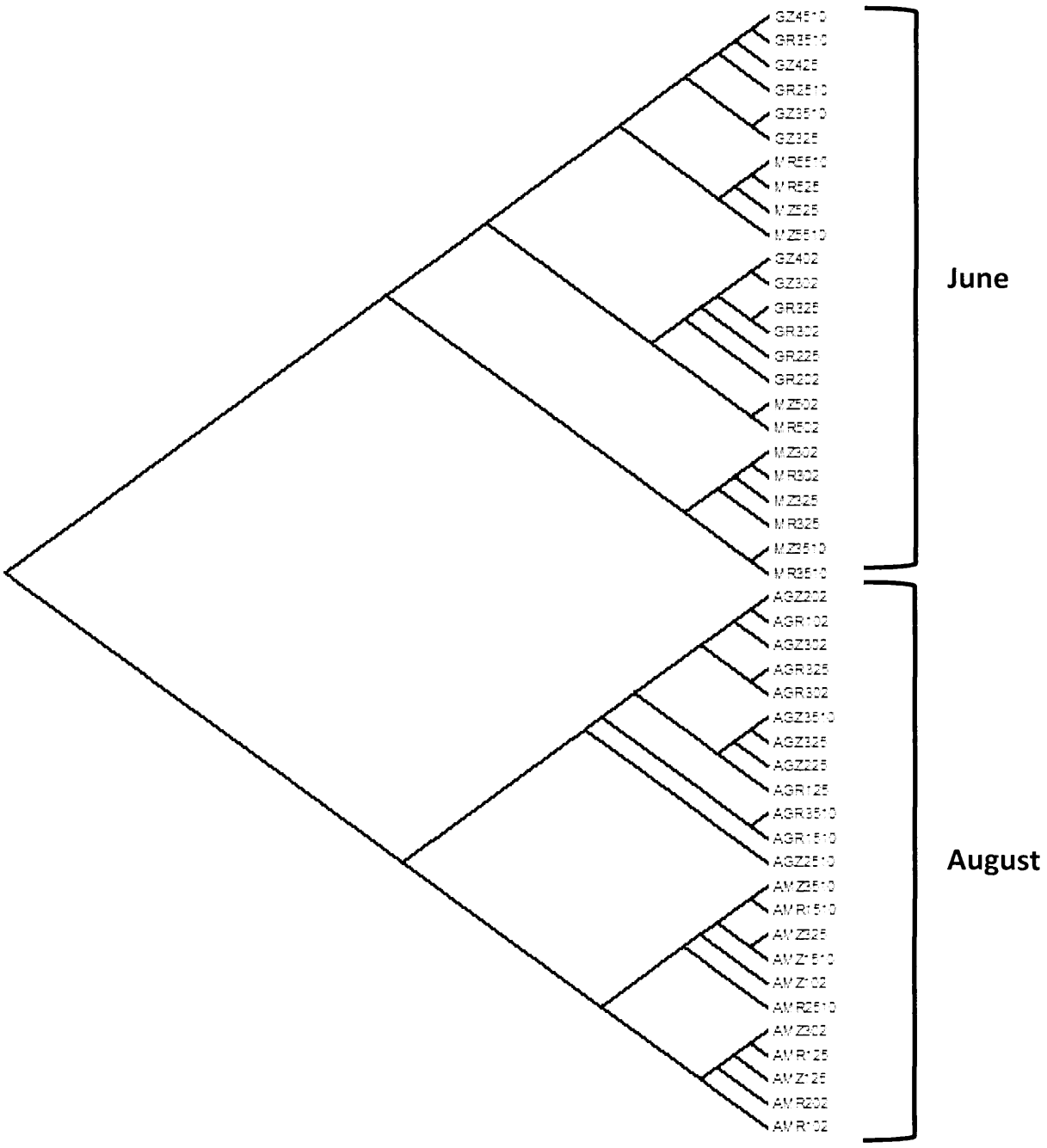

Figure 1. Cladogram of relatedness among microbial community samples. Samples are clustered by month and by site, month clustering is shown. Naming convention for samples includes, in order, the month for August samples (A), site (G for Goodwin, M for Mobjack), replicate number, and depth (0-2, 2-5, 5-10).

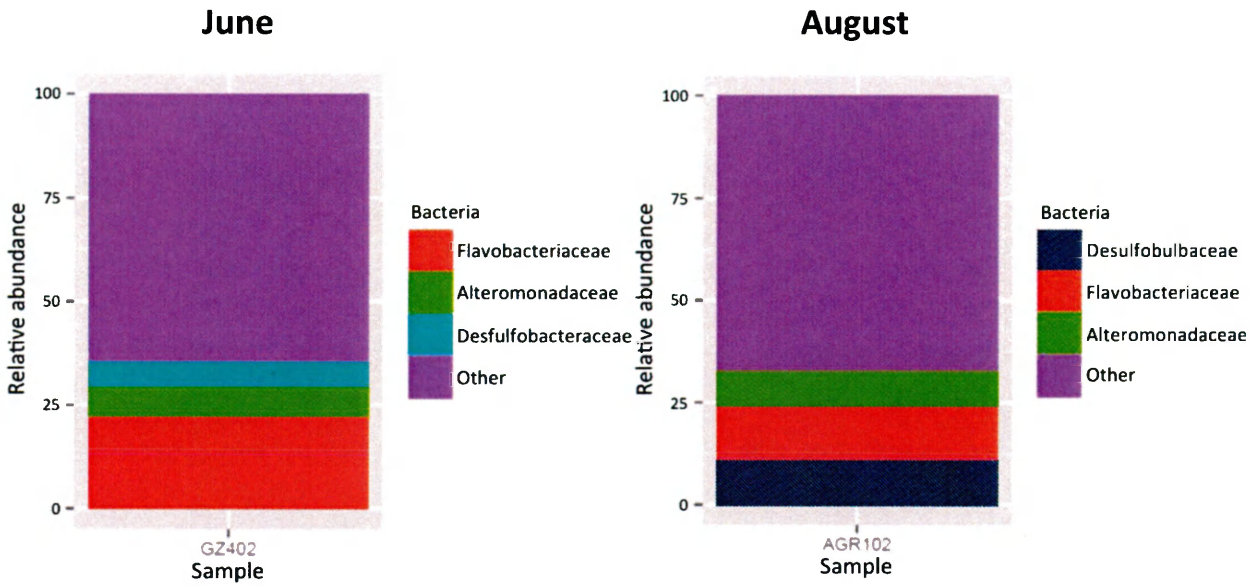

Figure 2. Representation of 5% relative abundance of bacterial families in samples from the Goodwin site during June and August. The August sample has higher than 5% relative abundance of *Desulfobulbaceae*, a sulfate-reducing family.

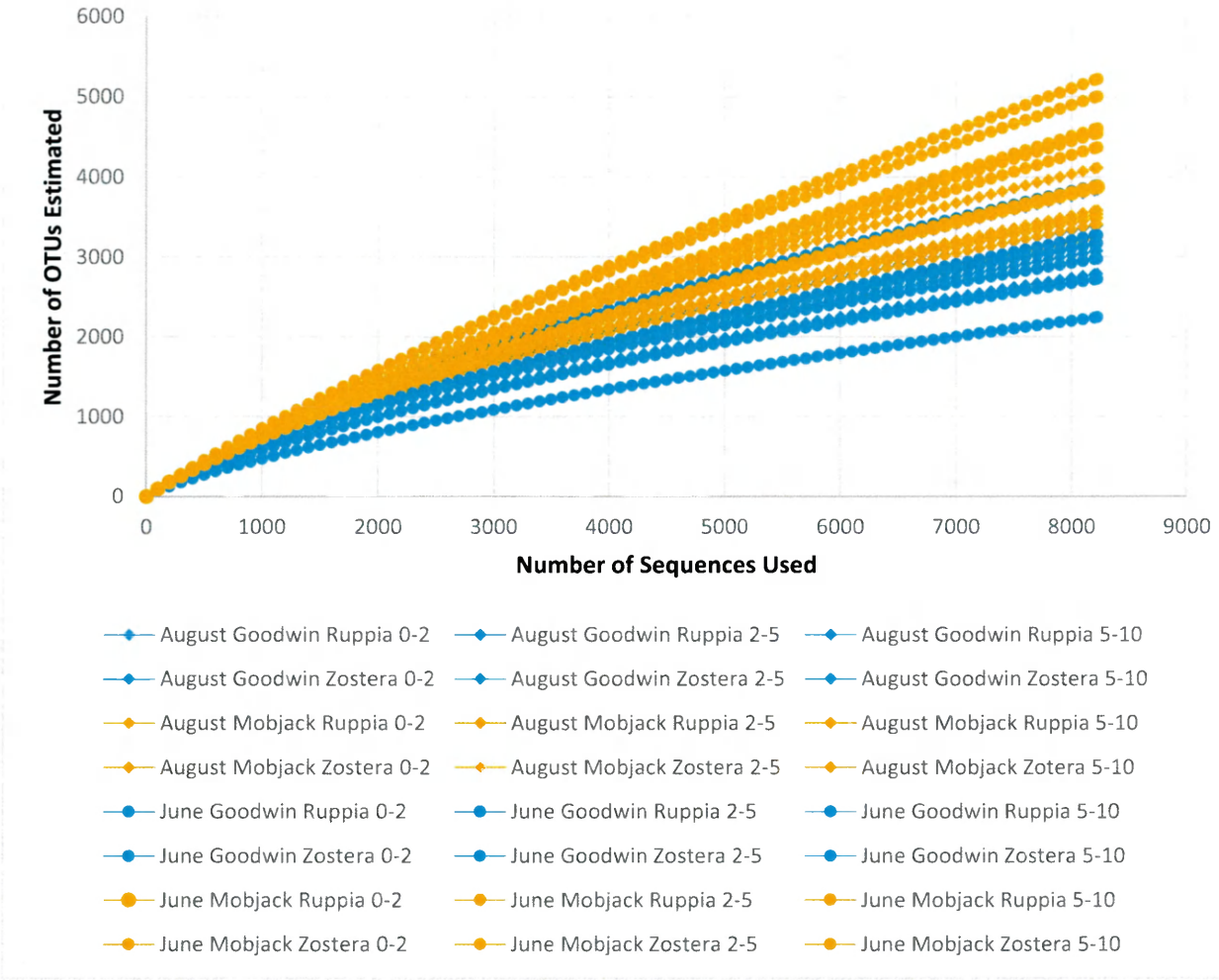

Figure 3. Rarefaction curve. Months are differentiated with symbology, sites are differentiated with colors. Mobjack sediments had higher bacterial species richness than Goodwin sediments.

Biomass

Similar to the above ground biomass results presented in Chapter 1, mean below ground biomass declined at all sites from June to August (Figure 4). Across the sites, *Z. marina* habitats showed a 71% decline in biomass from June to August, while *R. maritima* habitats declined 55% and mixed areas declined 75% (Table 2).

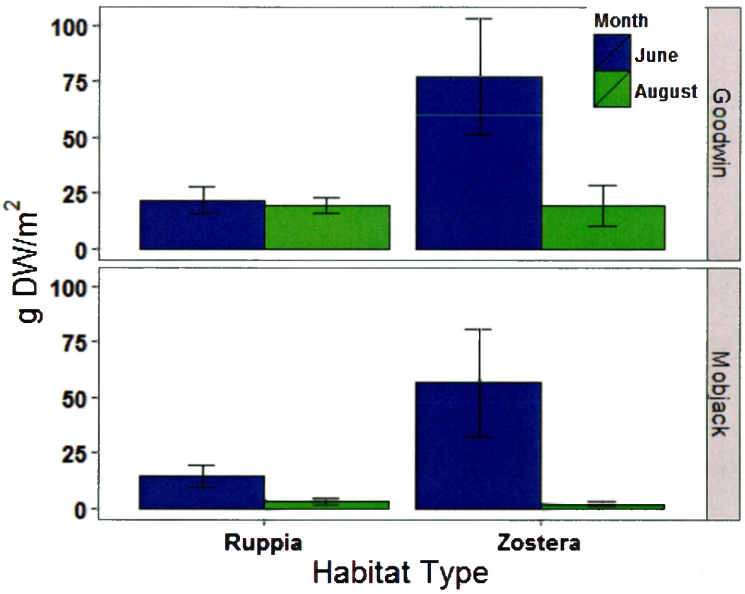

Figure 4. Mean below ground biomass by month, site and habitat type (N=5).

Month	Site	Mean site biomass (g DW/ m^2)	Habitat type	Mean habitat type biomass (g DW/ m^2)
June	Goodwin	45.74709	*R. maritima*	21.93023
			Mixed	38.25891
			Z. marina	77.05214
	Mobjack	31.27203	*R. maritima*	14.97696
			Mixed	22.001
			Z. marina	56.83812
	Poquoson	51.99259	*R. maritima*	33.77359
			Mixed	80.25454
			Z. marina	41.94963
August	Goodwin	19.26451	*R. maritima*	19.40016
			Mixed	18.67475
			Z. marina	19.71863
	Mobjack	2.35371	*R. maritima*	3.113933
			Mixed	1.466667
			Z. marina	2.480531
	Poquoson	15.34556	*R. maritima*	9.439109
			Mixed	14.81772
			Z. marina	21.77984

Table 2. Table shows means of below ground biomass collected over two months at three sites and three habitat types.

Sediment Characteristics

Across both months, the effect of site was significant on percent organic matter within sediment; Mobjack had higher organic matter than Goodwin (Figure 5). Within June and August, Mobjack also had higher sediment organic matter than Goodwin ($p<0.001$ and $p<0.005$, respectively). Additionally, sediment organic matter increased between June and August, across sites, habitat types and depths ($p<0.005$, Table 3). Organic matter did not have any correlation to microbial community diversity (Table 5).

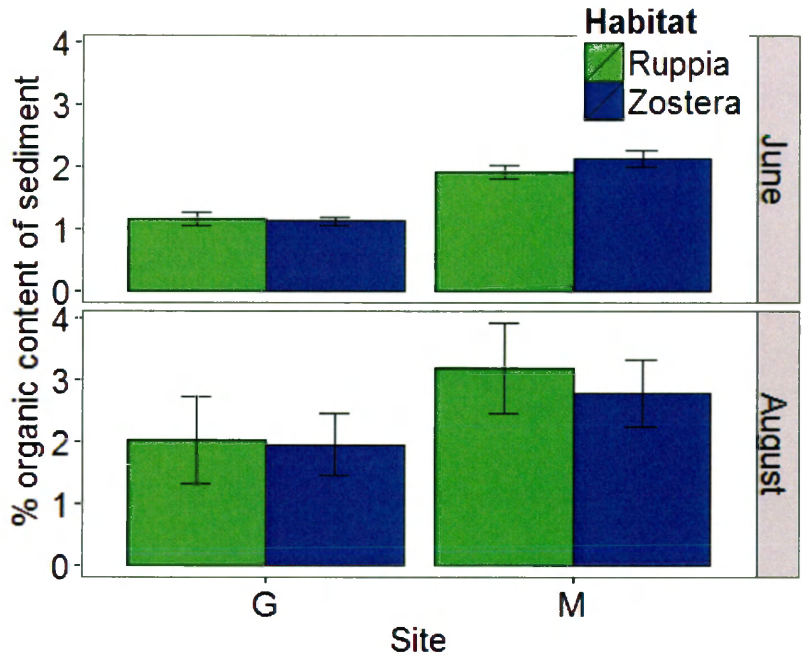

Figure 5. Mean organic content by month, site and habitat type. Organic content was significantly higher in the Mobjack site than the Goodwin site, and in the month of August versus June.

Effects	F-value	P-value
Month	8.2070	**0.005159**
Site	8.9575	**0.003539**
Treatment	0.0007	0.979861
Depth	2.8342	0.063854
Month: Site	0.0050	0.943753
Month: Treatment	0.1100	0.740856
Site: Treatment	0.0080	0.928749
Month: Depth	3.3020	**0.041169**
Site: Depth	0.5527	0.577294
Treatment: Depth	0.1474	0.863174
Month: Site: Treatment	0.1302	0.718922
Month: Site: Depth	0.6669	0.515741
Month: Treatment: Depth	0.1730	0.841423
Site: Treatment: Depth	0.2392	0.746523
Month: Site: Habitat: Depth	0.1694	0.844405

Table 3. ANOVA table for sediment organic matter (n=5 in most cases).

There was no effect of habitat type on ammonium concentrations. Sediment pore water NH_4^+ increased from June to August ($p<0.001$, Table 4). The effect of depth was significant across months, sites and habitat types ($p<0.001$); in most cases pore water NH_4^+ increased with depth. Interestingly, NH_4^+ did have a positive correlation with microbial community diversity (Table 5).

Effects	**F-value**	**P-value**
Month	47.7095	**1.843e-09**
Site	3.4998	0.06556
Depth	37.5452	**8.338e-12**
Habitat	2.4130	0.12848
Month: Depth	1.0147	0.36779
Site: Depth	0.0554	0.94618
Month: Habitat	0.1781	0.67427
Site: Habitat	0.1501	0.69960
Depth: Habitat	0.2271	0.79741
Month: Depth: Habitat	0.0607	0.94114
Site: Depth: Habitat	0.6981	0.50096

Table 4. ANOVA table of sediment ammonium.

Diversity indices	Sediment organic matter	Sediment ammonium
Shannon	r=0.059, p=0.694	r=0.249 p=0.143
Inverse Simpson	r=0.072 p=0.632	**r=0.320 p=0.057**

Table 5. Pearson correlation coefficients and p values.

DISCUSSION

The hypothesized differences between *Z. marina* and *R. maritima* sediment microbial community composition and bacterial family richness and diversity were not supported. There are several physiological explanations as to why microbial community differences were not seen between the habitat types. It is possible that light to *Z. marina* was attenuated by epiphytes growing on the blade surfaces, causing it to photosynthesize less and consequently pump less oxygen down through its roots (Kristensen et al. 2005) making the sediment environment more similar to *R. maritima* stands which typically have lower belowground biomass. *Z. marina* aboveground material per unit area has a higher surface area than *R. maritima*, and also has strap-like leaves, as opposed to *R. maritima*'s rounded leaves (personal observation), which provides more surface area for epiphytes. This could have an equalizing effect in terms of nutrients, organic matter and oxygen pumped down into the sediment between *Z. marina* and *R. maritima*. Interestingly, Jovanovic et al. (2015) recently reported *R. maritima* has greater radial oxygen loss from its roots than *Z. marina*, and concluded that it could be an important adaptation for living in sediments with high levels of organic matter. This feature, too, could have an equalizing effect for the two seagrass root exudates, and could explain why differences between microbial communities were not evident.

The main effects of month and site were significant in this study. The decrease in mean root biomass from June to August likely affected community composition, by decreasing root exudates and increasing sulfate reduction and thereby sulfate-reducing bacteria (Holmer and Bondgaard 2001). This was reflected in the increase of relative abundances of the family *Desulfobulbaceae* in the August samples. Accumulation of allochthonous organic matter, as well

as senescing leaf material from throughout the summer, may have contributed to the observed increase in organic matter and pore water ammonium in the sediments at both sites from June to August.

Although community composition data showed the most differences by month, diversity and richness data differed by site. Both bacterial species richness and diversity were significantly higher at Mobjack than Goodwin, across and within months. Although organic matter also exhibited the pattern of being higher at Mobjack than Goodwin both across and within months, when regressed with diversity indices, they did not have any correlation. Sediment ammonium did have a marginally significant relationship with one of the diversity indices. A greater amount of organic matter at Mobjack than Goodwin could be responsible for more decomposition, higher bacterial diversity, and consequently greater pools of ammonium as a result of decomposition.

The lack of differences between the species observed here suggests that the two seagrass sedimentary environments were similarly affected by the vegetation during this period of study, and so had similar bacterial communities. However, before assuming this is always the case, more site- specific research should be done to see if it is possible to better elucidate the potential for habitat specific differences. As *Z. marina* declines in the Chesapeake Bay and is replaced by *R. maritima* in some areas, or *R. maritima* is considered as a restoration option in some areas of the Chesapeake Bay and elsewhere, it is important to better understand if the two seagrasses possess similar microbial communities and, as a result, provide potentially similar nutrient cycling capabilities.

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